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THE EFFECT OF FARADIC AND GALVANIC STIMULATION UPON THE COURSE OF ATROPHY IN DENERVATED SKELETAL MUSCLES

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Although electrotherapy has been used for more than a century by the medical profession to improve the power of paralyzed muscles, there still exists an extensive clinical controversy about the value of electrotherapy. Today, even the use of electrotherapy in cases of lesions of the motor nerves is praised by some and regarded as useless by others. Despite this clinical dispute, almost no experimental data can be found in the literature to support either point of view. Langley and Kato (1915) stimulated in five rabbits the denervated gastrocnemius-soleus muscle with a few hundred condensor discharges daily and found that the treated muscle lost a little less weight than the untreated control muscles. Langley (1916) reported experience with one rabbit stimulated by condensor discharges for about two hours daily. After 21 days the treated denervated gastrocnemius-soleus showed less fibrillation, was about 5 per cent heavier, and had a higher electrical excitability than the untreated denervated muscle of the other hind leg. Langley and Hashimoto (1918) found that galvanic treatment at least diminishes fibrillation of the denervated muscle. Hartman and Blatz (1920) reported experiments on 24 rabbits, in which they used for 15 minutes daily submaximal stimulation by direct current, interrupted rhythmically by a metronome. They compared only the power of the treated and untreated denervated gastrocnemius muscles and observed no distinct beneficial effect of this electrical treatment in preventing the degenerative processes or in assisting the recovery produced by the regeneration of the motor nerve.

In nearly all these and earlier animal experiments weak electrical stimu-

lation with single excitations was used, since the prevailing view of the clinicians was and is in favor of weak electrical treatment. Besides, on account of the observed spontaneous fibrillations in denervated muscles Langley (1916) emphasized the view that the atrophy is not a disuse atrophy but a fatigue atrophy. The clinicians too usually explain their observed beneficial effect of electrotherapy much more by an influence upon the excitatory mechanism than upon the contractile mechanism of the muscle. However, Langley himself realized that by the use of a weak current, only the superficial fibers of the denervated muscle can be influenced. Since in all these investigations the strength of the contractions produced was considered more or less unimportant, no attempt has been made to modify the character of the electrical stimulation according to the well known changes in excitability during progressing atrophy. For clinical purposes this point has been emphasized by Walthard (1935), and he proposes the use of frequent "chronaxie stimulations."

The preference of weak and ineffective stimulation for therapeutic use is astonishing, if one regards the fact that all investigators, who reported training effects on normal muscles after daily electrical treatment, used strong currents. In such experiments not only marked increases in muscle weight and power (Debedat, 1894; Bordier, 1902) but also increased glycogen content (Embden and Habs, 1927), increased resynthesis capacities (Habs, 1927; Guckelberger and Keiser, 1938) and other metabolism increases and changes (Werbolowitsch, 1937) have been observed.

It seems reasonable to assume that, as in normal muscle, also in denervated muscle strong stimulation provoking vigorous contractions may produce something like a training effect and perhaps in that way counterbalance atrophy to a certain extent. Therefore, experiments with repeated daily strong faradization and galvanization were carried out on a large number of rats. The influence upon excitability, weight, water content, O_2 consumption in vitro and upon birefringence was determined for the normal and the denervated gastrocnemius-soleus muscle.

MATERIAL AND METHOD. In rats, 4 to 6 months old, one or both sciatic nerves were resected aseptically as centrally as possible for at least a length of 1 cm. The operated rats were kept in single cages and fostered carefully to avoid the occurrence of edema and of so-called trophic ulcers. Despite all care, some of the animals had to be discarded on account of such lesions. These lesions were mostly bilateral ones in rats operated on both sides (Fischer, 1939). Since Holobut and Jalowy (1936) found that the regenerating nerve fibers can reach the denervated rat gastrocnemius only after 28 days, all rats, which were kept for four weeks, were reoperated after 23 to 26 days.

Stimulation. According to the plan in the various experimental series, the gastrocnemius-soleus muscle of one or of both sides was stimulated

daily for a period of 12–20 minutes. Since strong currents were used, the animals were kept during stimulation under slight ether anesthesia. The only observed effect of the frequent narcosis was a constant small loss in body weight. The current used for stimulation was either tetanizing current from an induction coil or interrupted galvanic current from dry cells. The interruption was produced by a motor-driven mechanical device, allowing 8 to 20 interruptions per second. The intervals between the rectangular current shocks were of the same durations as the shocks. The interruption frequency was adjusted to the need of the particular muscles to produce the strongest possible contractions with a current as weak as possible. With progressing atrophy, this frequency has to be diminished, which means also an increased duration of each single current shock. In this way it was possible to produce even in a muscle with marked atrophy tetanic contractions. A second automatic interruptor was used during faradic as well as galvanic stimulation for turning the current on and off each half second. Thus 60 short but strong contractions were produced per minute. After 2 to 3 minutes of stimulation a rest period of 1 to 2 minutes was interpolated and then the direction of the current changed. The strength of the current was constantly adjusted to give optimal contraction without any unnecessary overstimulation. One electrode, consisting of saline-soaked gauze, was wrapped thickly around the hind-foot and covered by a thin lead sheet. The other electrode was a saline-soaked wad of absorbing cotton pressed slightly by a spring against the belly of the gastrocnemius (surface of contact about 1 sq.cm.).

Measurement of excitability. With the usual condensor arrangement the strength-capacity curve was determined for just visible contractions. In some experiments only the chronaxie was measured. As cathode on the belly of the muscle, an electrode similar but smaller ($\frac{1}{2}$ sq.cm.) than that for stimulation was employed.

Weight and dry substance determination. Since stimulated muscles for some hours after work are swollen and contain more water than normally (Fenn et al., 1938; Boyle and Scott, 1938), the rats were killed 24 to 36 hours after the last stimulation period by a blow on the head and were bled from the carotids. Special care was taken to dissect out and clean both muscles to the same extent from other tissues and to cut the tendons in exactly corresponding positions. The muscles were weighed immediately and a small part was cut out for use in the respirometer. Then the muscles were weighed again and dried at 108°C. to a constant weight.

Oxygen consumption. The Thunberg-Fenn respirometer was employed. A small bundle of muscle fibers of each gastrocnemius (from corresponding parts) was weighed (40 to 80 mgm.) and put in a watch glass with oxygenated buffered Tyrode's solution. The bundle was then carefully teased out into thin fibers, and finally the fibers transferred with 2 cc. Tyrode's

solution into the experimental bottle of two identical respirometers and suspended and shaken in a constant temperature bath at 37.5°C. After an hour the index drops reached more or less constant speeds. The average speed maintained for the next hour was used for the calculation of the oxygen consumption in terms of O_2 used per gram tissue (dry weight) per hour.

Measurement of double refraction. Some of the birefringence measurements were done on the muscles of rats not used for other determinations, and the whole muscles were weighed and then fixed at resting length in 10 per cent formaldehyde Tyrode's solution. Were the muscles also used for other determinations, only parts of the muscles were fixed. After fixation, single fibers were carefully isolated, total birefringence measured, and micellar birefringence determined by the imbibition method (Noll and Weber, 1935; Fischer, 1936a, 1938). For each muscle the determinations were carried out on 4 to 6 fibers and the average values calculated.

Results. *Excitability.* Muscular excitability differed markedly from one rat to the other, but the normal left and right gastrocnemius of the same animal, when under slight ether narcosis, had always about the same strength-capacity curve (table 1A). The difference between the chronaxies of matching normal muscles was in no instance over 15 per cent when electrodes of the same size had been placed carefully on identical spots. Even the changes of the chronaxie of normal muscles, when measured day by day, are very small (not more than 20 per cent) as long as narcosis is carefully administered each day to the same depth in about equal times (left end of fig. 1).

During the first 2 or 3 days after nerve section the chronaxie lengthens very slowly. At that time the average difference in chronaxie between the denervated muscle and its normal partner is not greater than the maximal difference observed between matching normal muscles. Only between the third and ninth days, the lengthening of the chronaxie of the denervated muscle becomes so pronounced that it can be seen without statistical methods. This is illustrated by table 1B, which represents the findings on the sixth day after denervation for that rat, in which these changes developed the most slowly of the whole series. Nevertheless, the chronaxie has lengthened about 50 per cent, and on the next day this difference was already more than 100 per cent. A doubling of the chronaxie values was reached in all cases between the fifth and seventh days and the chronaxie further lengthened with progressing atrophy, at first at about a constant rate (fig. 1), but later on at a somewhat diminished rate. The maximal lengthening of chronaxie observed in rats kept alive more than five weeks after denervation was between 120 to 185 times.

Since these experiments revealed from the beginning that the excitability changes develop with various speeds in different rats, both sciatic nerves

were cut simultaneously in some rats and the chronaxies of both gastrocnemius-soleus muscles were measured during the course of atrophy. In these rats always a fairly uniform rate of chronaxie lengthening was observed for the matching muscles. The maximal differences observed were not more than 18 per cent during the first two weeks, while later on with marked lengthening of the chronaxies the differences occasionally reached almost 25 per cent. Therefore, it was decided to test the effect of the electrotherapy by stimulating one muscle in rats with both gastrocnemii denervated. Table 1C gives the data obtained from a rat, operated seven

TABLE 1

Strength-capacity values and chronaxie (in msec.) for normal and denervated rat gastrocnemii

A. Normal left and right muscle

	μF	1.00	0.50	0.25	0.15	0.100	0.075	0.061	0.054	0.050	0.045	0.040	0.035	0.030	CHRONAXIE
Left normal	Volt	13.1	15.2	17.1	17.9	19.9	22.3	26.2		31.0	35.7	45.8	58.8	79.0	0.56
Right normal	Volt	14.8	16.5	19.0	20.1	21.4	24.3		29.6	34.7	39.0	51.5	68.4		0.50

B. Left normal muscle, right muscle denervated six days previously

	μF	1.00	0.50	0.25	0.15	0.108	0.100	0.075	0.050	0.030	0.020	0.010	CHRONAXIE
Left normal	Volt	21.0	23.9	29.5	31.9		38.9	42.0	48.1	52.0	58.3	71.2	0.69
Right atroph	Volt	19.1	22.8	31.0	33.6	38.2	42.0	50.8	51.0	68.5			1.00

C. Left as well as right muscle denervated seven days previously, right muscle stimulated faradically on the 4th, 5th and 6th days

	μF	1.00	0.50	0.25	0.225	0.200	0.191	0.175	0.150	0.130	0.110	0.090	0.075	0.065	CHRONAXIE
Left atroph	Volt	13.9	17.0	19.5	19.7	20.3		20.9	21.4	27.8	37.3	48.7	60.3	79.7	1.20
Right atroph exerc.	Volt	15.8	21.8	25.6	26.4	29.0	31.6	39.8	49.8	68.8					1.76

D. Normal left and normal right muscle, the latter stimulated faradically the last five days

	μF	1.00	0.50	0.25	0.15	0.100	0.095	0.080	0.070	0.067	0.060	0.050	CHRONAXIE
Left normal	Volt	27.6	29.1	31.4	36.4	41.7		45.4	51.8	55.2	60.3	71.7	0.62
Right normal exerc.	Volt	29.8	31.8	38.6	46.8	56.7	59.6	69.2	81.7				0.88

days previously, and its right gastrocnemius faradically treated on the fourth, fifth, and sixth days. In this and in all corresponding experiments, the excitability was further decreased by such a short treatment. Since Laugier and Neoussikene (1933) reported that in human muscles after work provoked by electrical stimulation, the chronaxie will be lengthened for a considerable time, it was likely that in rat, independent of atrophy, a similar excitability depressing effect of faradic current exists. Table 1D, giving the data for a normal muscle pair, one of which was stimulated the five preceding days, reveals that this suspicion was correct.

Nevertheless, prolonged faradic stimulation of a denervated muscle has a beneficial effect upon the excitability. This is well illustrated by the experiment represented in figure 1, in which nine days of electrical exercise was finally able to shorten markedly the chronaxie of the treated muscle in comparison to that of the untreated. Two days later the rat was killed and the values found for weight, relative dry weight, and for O_2 consumption of the treated muscles were markedly higher than those of the untreated one. In all experiments in which faradic stimulation was used for the first 7 to 13 days of atrophy, and in those longer experiments, in which

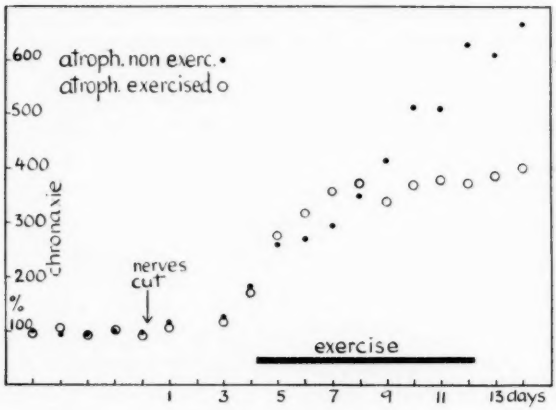

Fig. 1

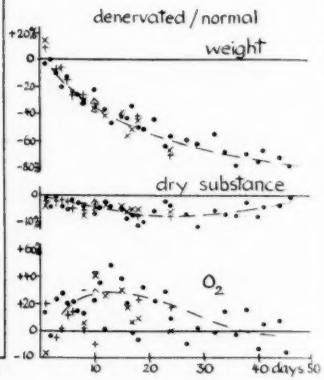

Fig. 2

Fig. 1. Daily changes in chronaxie in a pair of denervated muscles; the right, daily exercised for nine days. At death of the rat (15 days after denervation) the weight of the exercised muscle was 1.034 grams, dry substance 23.48 per cent, and O_2 grams dry weight per minute 4.6 mm.^{3} The corresponding values for the untreated muscle were 0.860 gram, 22.80 per cent, and 4.2 mm.^{3}

Fig. 2. Loss of weight, loss in relative dry substance, and increase in O_2 consumption of denervated muscles in comparison with their normal partners.

later on galvanic stimulation was employed, corresponding effects were observed. The chronaxie is increased during the first days of treatment, but later the chronaxie of the treated muscle lengthens rather slowly, while in the untreated muscle the chronaxie rapidly lengthens.

If a denervated muscle has been left untreated for about two weeks or more, faradic stimulation no longer produces any appreciable contraction. However, galvanic stimulation at that time provokes contractions, and if repeated daily, the further lengthening of the chronaxie will be delayed to some extent, but not nearly so markedly as with early treatment by faradic current.

Weight, dry substance content, and O_2 consumption in vitro. Due to the large variation in the rate of atrophy, as revealed by preliminary experiments, it was necessary to establish at first on a relatively large number of animals, the average course of atrophy in untreated muscle, and to find out how much atrophy varies in matching muscles. Figure 2 summarizes the changes concerning muscle weight, percentage of dry substance, and O_2 consumption with increasing time of atrophy. All data are expressed as

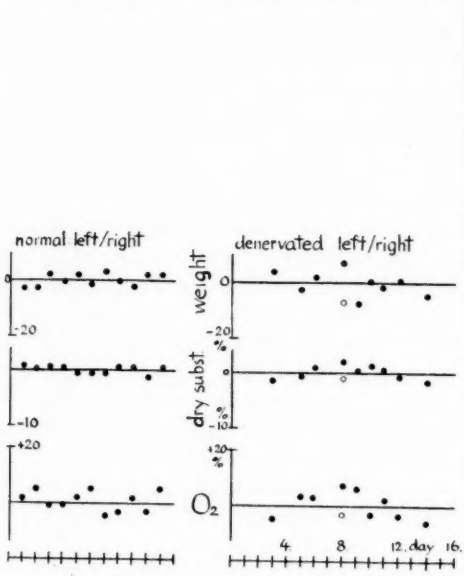

Fig. 3

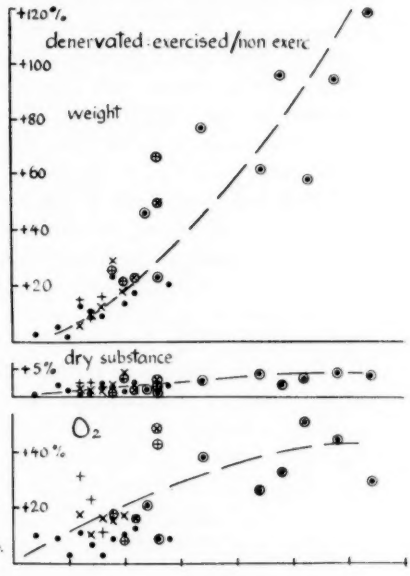

Fig. 4

Fig. 3. Comparison of weight, dry substance, and O_2 consumption between normal partners (left) and denervated partners (right).

Fig. 4. Comparison of weight, dry weight, and O_2 consumption between denervated exercised muscles and their non-exercised denervated partners. The encircled points refer to experiments in which, besides faradic currents, galvanic currents were also used (after 7 to 13 days), and finally only galvanic currents (from the fourth week). Abscissae in five days intervals.

the difference in per cent between the denervated muscle and its normal partner. Although a large scattering of the experimental points is evident, the curves demonstrating weight loss and diminished dry substance content can be drawn fairly accurately. Although the scattering for the O_2 values is rather large, it may be safe to say that very soon after denervation the metabolism tends to increase, that a maximum is reached after about two weeks, and that then the metabolism again reaches a more normal

level. The scattering of the points for the three types of measurements, especially of those for the O_2 consumption, is not caused by the methods employed, as can be seen from figure 3, which represents the results of control experiments with matching normal and with matching denervated muscles. The left muscle is always compared with the right. The scattering of the points is only a little larger for the denervated muscles than for the normal ones. Thus the marked deviations from the general curves found when comparing an atrophying muscle with its normal partner must be due to variation in the course of atrophy from animal to animal. But as for the excitability changes, the control experiments with both muscles denervated indicate that in the same animal the rate of atrophy is about equal in both hind-legs.

In consequence of all this, any larger difference between treated and untreated partner of a denervated muscle pair must be attributed to the electrotherapy. Figure 4 gives a summary of the results concerning weight, dry substance, and O_2 consumption. All values are expressed in per cent increase of the treated above the untreated muscle. All the data are from rats, which had been treated daily from the first day after denervation until 24 to 36 hours before the animals were killed. Up to about the tenth day only faradic stimulation was used. From that time on the muscles were stimulated by faradic and galvanic currents, or by the latter alone. The apparent gain in weight and relative dry weight of the treated muscles is quite large. But it will be pointed out later that nevertheless the treated muscle is far under normal weight and normal percentage of dry substance. The excess O_2 consumption (per gram dried muscle) of the atrophying muscle is not diminished by the treatment, but on the contrary is still further increased.

Control experiments (fig. 5a) on normal muscle pairs revealed that faradic stimulation also increases their weight, dry substance, and O_2 consumption. However, galvanic stimulation tends to decrease the weight of normal muscles, while relative dry weight and O_2 need are only slightly and irregularly influenced (fig. 5b). That with denervation and progressing atrophy the reaction of the muscle against these two types of electrical stimulation changes, is demonstrated by a small series of rats with both sciatics cut, and then one gastrocnemius treated galvanically and the other faradically (fig. 5c). While in the main series treatment started for all animals directly after denervation, in these rats the treatment was of about equal duration (7 to 9 days) but started at various times after operation. The results are expressed as excess or loss of the galvanically stimulated muscle in comparison to the faradically treated. The data demonstrate distinctly that faradic current is more beneficial at the early stage of atrophy, but the galvanic current is preferable in the later stage.

Since in the experiments represented in figure 2 the general course of

atrophy has been established, and since in the experiments of figure 4 the relation between the relative beneficial effect of electrical exercise and the duration of treatment has been found, the general course of atrophy for treated muscles can be calculated and compared with the atrophy course in the untreated muscle (fig. 6). It is evident that the electrical treatment employed is unable to prevent weight loss, but retards it markedly, so that finally continued treatment can almost arrest further weight loss at least

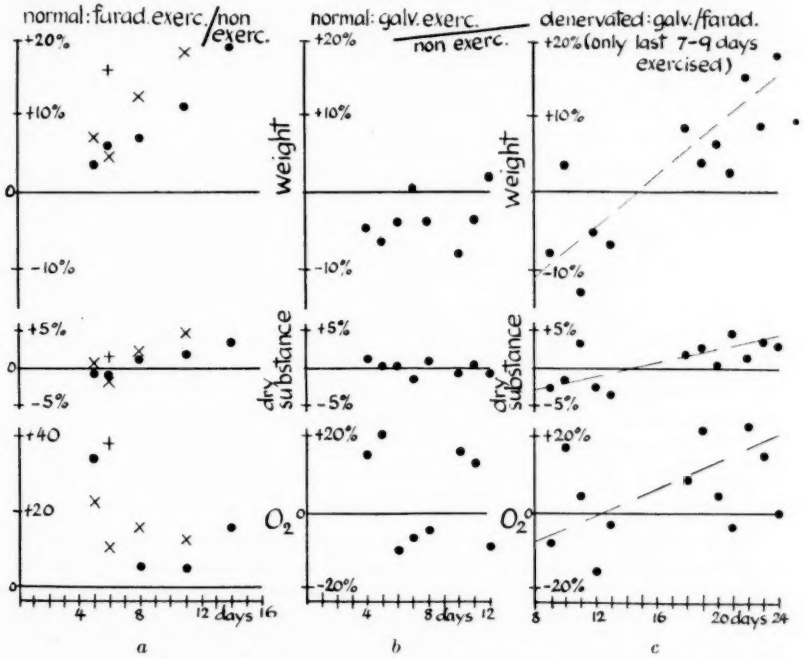

Fig. 5. Comparison of weight, dry substance, and O_2 consumption of matching muscles: *a*, between faradically exercised normal muscles and their non-exercised normal partners; *b*, between galvanically exercised normal muscles and their non-exercised normal partners; *c*, between galvanically exercised denervated muscles and their faradically exercised denervated partners. Duration of treatment was constant in *c*, but started at various times after denervation.

for the time period investigated. Also the loss in relative dry weight is markedly retarded. However, the slight secondary increase in relative dry weight occurring from the third week on, is probably not due to the exercise, since such a secondary increase, even more distinct, is observed also in the untreated muscle. The secondary increase of relative dry weight in the untreated muscle is probably due to the marked proliferation of connective tissue at that phase of atrophy (Tower, 1935).

Birefringence. Measurements were carried out on three normal rats with one hind-leg exercised for about two weeks, on four rats with one sciatic nerve cut, and on eight rats with both hind-limbs denervated and one side stimulated daily. No difference in birefringence was found between normal exercised and non-exercised muscles. The differences in the measured values for various fibers of the muscles were much larger in denervated muscles than in normal muscles, thus indicating that the course of atrophy varies even from fiber to fiber. Figure 7 represents the experimentally found data for total birefringence and micellar birefringence, and the calculated data for form birefringence. In order to get a clear graph, the data for the ten normal muscles are not represented by single points.

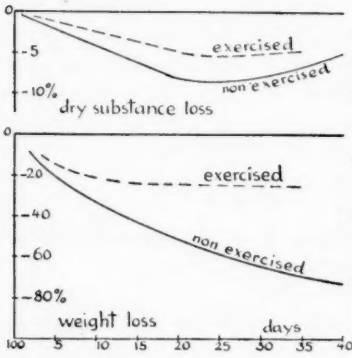

Fig. 6

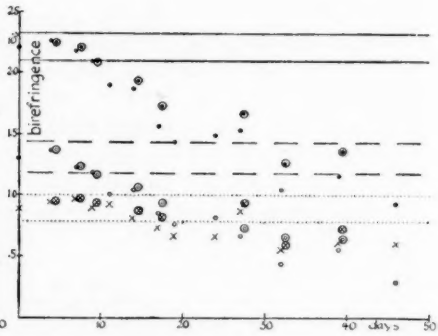

Fig. 7

Fig. 6. Comparison of the general course of atrophy as measured by the loss in weight and in relative dry weight for exercised and non-exercised denervated muscles.

Fig. 7. Total birefringence (dots), form birefringence (circles), and micellar birefringence (crosses) of treated and untreated denervated muscles. The points for the treated muscles are encircled and shifted a little to the right in order to avoid overlapping. The average values for ten normal muscles are given at zero time, and the observed ranges of deviation are indicated by horizontal lines.

Only the average values for all normal muscles are given (at zero time) and the range of the observed deviations indicated by parallel horizontal lines. The points for the treated denervated muscles are encircled and shifted a little to the right from the points of their untreated partner, since otherwise in many instances overlapping of the points would occur. Only about ten days after denervation a loss in total birefringence becomes appreciable. After 6 to 7 weeks total birefringence will be about half its original value. This loss in total birefringence is mainly due to a diminished form birefringence, while micellar birefringence is much less altered. Electrical treatment has, however, little beneficial effect upon these birefringence changes compared with its marked effect upon the weight loss.

Using the imbibition method for determining the micellar birefringence, the minimum double refraction was found both for normal and denervated muscles with fluids of refractive indices between 1.55 and 1.60.

DISCUSSION. Hines and Knowlton (1933) pointed out that in rats atrophy occurs much faster than in men and the larger experimental animals. They observed too that during atrophy the water content increases (1937). Tower (1935, 1939) emphasizes that not only the rate of atrophy differs from one species to the other, but that even in the same animal various muscle groups undergo atrophy at different speeds. She reports for the m. interosseus of the cat, a muscle which loses its weight relatively fast, a weight loss of about 50 per cent in four months. According to Langley and Kato (1915) rabbit gastrocnemii atrophy to half their weight in 6 weeks, while in the experiments reported here, the rat muscles reached this stage of atrophy between the 16th and 20th days.

Probably similar differences concerning excitability-loss exist. Richter (1936), who investigated only the first 48 hours, found a small lengthening of chronaxie in his rats only near the end of that period. Holobut and Jalowy (1936), who were primarily interested in the excitability changes of the degenerating rat nerve, found a distinct lengthening of the chronaxie of the muscles between the third and fifth days. While all these data are in accord with the results reported here, Adrian (1916) observed a change in strength-duration curve of denervated human muscles only after the eighth day. This difference in speed of excitability-loss is probably the reason why in my experiments broken strength-capacity curves, made up by the time-constant of still excitable nerve fibers and the time-constant of the muscle fibers themselves, were observed only during the first few days. Apparently also the type of electrodes used and their location were not favorable for obtaining broken curves, since such curves were obtained only occasionally, and were often not obtained any longer after a small shifting of the electrodes. The maximal lengthening of the chronaxie observed by Adrian was 40 times, which is in agreement with the reports of 30 to 40 times for man by Bourguignon (1923), while the latter (1925) reports 100 to 200 times as maximal lengthening of chronaxie for dogs. In rabbits Kosaka (1935) found a lengthening of 50 to 130 times, which is less than the 120 to 185 times observed in those rats kept longer than 5 weeks. According to these figures there exists no relation between the speed of atrophy and the maximal lengthening of chronaxie in different species. However, no conclusion can be drawn, since most of the authors cited were interested in restitution of the muscles, and therefore did not reoperate upon their animals, thus permitting the growing nerve fibers to reinnervate the muscles.

The results reported here concerning the effect of vigorous electrical treatment, show distinctly that at least three typical symptoms of atrophy, namely, weight loss, increased water content, and diminished excitability

can be markedly retarded by stimulations adapted in their duration to the changed excitability. But the O_2 consumption of the denervated muscle is further increased by the treatment and not diminished as one might expect. Langley and Itagaki (1918) reported as one proof for Langley's fatigue theory of atrophy a triple increase in metabolism in denervated dog muscles measured by the arteriovenous oxygen difference. However, no changes in metabolism were found by similar methods used also on dogs by Hines, Leese, and Knowlton (1931). For the rat muscle in vitro, Knowlton and Hines (1934) found a small increase in O_2 consumption, when calculated per gram dry weight. Tschepinoga (1938) reports a marked increase in O_2 need for denervated rabbit muscles, and he observed, as in the experiments of this paper, a large variation in the size of metabolism of the denervated muscles.

The fact that electrical treatment decreases weight loss and increases water content brought about by denervation, but increases furthermore the quantitatively raised, but qualitatively impaired (Knowlton and Hines, 1934) metabolism, seems to afford a clue for the explanation of the beneficial effect of the treatment. This effect is identical with the training effect by electrical stimulation upon normal muscle, where also the weight increases and the metabolism is raised and improved in efficiency (Embden and Habs, 1927; Habs, 1927). There is no reason why such a training effect should not appear on the denervated muscle as long as the latter is still excitable. In frog muscles, in which, after denervation, excitability and structure remain almost constant for weeks, a marked prevention of weight loss by electrical treatment has always been observed since the first report of Reid (1841).

The training effect upon normal muscle is mainly upon the thickness of the muscle fibers and on the capacity of their metabolic mechanism, but until now nobody was able to demonstrate that the power of the muscle per square unit can be increased by the treatment. The same is more or less true for my experiments with denervated muscles. Although no measurements of the power of the muscles were carried out, it was obvious from mere observation and palpation that the increase in power only corresponds approximately to the increase in muscle substance and increased excitability. As seen from figure 4, a treated muscle five weeks after denervation has about the same weight as an untreated denervated muscle after about one week. Nevertheless, the contractions of the muscle, treated for five weeks, are rather powerless; while the power of an untreated muscle one week after denervation is still rather high. But compared with its untreated partner, the power of the muscle treated for five weeks is appreciably higher, and especially the treated muscle is less fatigable.

There exists apparently a parallelism between the power of a denervated muscle and its birefringence, since the latter diminishes appreciably only

after about ten days, and since electrical treatment has but little effect in retarding the birefringence changes.

The ultrastructure of the muscle fiber is regarded today as important for the contractile mechanism (Fischer, 1936b; Schmitt, 1939). Therefore, the fact that electrical treatment of the denervated muscle only improves to a small extent its impaired ultrastructure, indicates that despite the remarkable retardation in weight loss and diminished loss in dry substance, the treatment has failed to improve the contractile mechanism.

The observed loss in birefringence during atrophy can be discussed to a certain extent according to O. Wiener's theory (Fischer, 1936b; Schmitt, 1939). The remarkable loss in form birefringence with no change in the refractive index of the surrounding phase indicates that the relative volume of the micellae is diminished in comparison to the relative volume of the surrounding phase. However, since micellar birefringence is but slightly diminished, the loss in relative micellar volume cannot be due to a diminished relative number of the micellae. One must assume that the micellar volume is diminished due to a shrinkage of the single micellae with simultaneous impairment of the crystalline structure of the micellae.

Since no experiments were performed in which the regenerating nerve fibers were allowed to re-innervate the atrophied muscles, there is no direct evidence that a treated muscle after re-innervation will regain normal strength faster than an untreated one. However, it seems reasonable that a treated muscle with its higher excitability, its greater weight, its lower water content, and its larger metabolism, may more easily be restored to normal function than an untreated muscle.

SUMMARY

In rats with one hind leg denervated, the course of atrophy of the gastrocnemius-soleus muscle was investigated by determination of excitability, weight, water content, O_2 consumption in vitro, and total birefringence and micellar birefringence.

Corresponding determinations on rats with both legs denervated, but with one gastrocnemius stimulated every day vigorously with suitable electrical currents for a period of 12 to 20 minutes, revealed that such treatment is able to retard markedly the loss in excitability, and the loss in relative dry weight, while the already raised metabolism is further increased. However, the loss in birefringence is only a little diminished and the power of the treated muscle, although distinctly higher than that of the untreated, appears to be far less than one would expect, if the contractile mechanism of the denervated muscle were influenced by the treatment to the same extent as is the weight loss. The loss in total birefringence is mainly caused by a loss in form birefringence.

Experiments with electrical treatment starting at various times after

denervation demonstrated that the best results are obtained with a treatment starting immediately after denervation, and that the strength and the duration of the electrical currents must be adapted to the changing excitability of the atrophying muscle.

It is postulated that the electrical treatment causes mainly a training effect in the denervated muscle, similar to that in normal muscle, which only increases the size of the fibers and their metabolic capacity, but does not influence markedly the ultrastructure of either denervated or normal muscle. The failure of the electrical treatment to repair the damaged ultrastructure of the denervated muscle must be regarded as the main reason why, despite the marked arrest of atrophy as measured by the relative weight increase and increased excitability, the treated denervated muscle appears to improve only slightly in power.

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PHYSIOLOGICAL DELIMITATION OF NEURONES IN THE CENTRAL NERVOUS SYSTEM[1]

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Heretofore for the delimitation of neurones in the central nervous system (CNS) the only methods available have been anatomical: the Marchi method, the Weigert method and the method of retrograde cell degeneration. Although fully recognizing the admirable services these methods have rendered, one should not be blind to their limitations. These methods are time-consuming and require in experimentation sterile operative procedures with a survival of the animal for weeks or even months. These are only small practical disadvantages; heavier weigh the theoretical objections to these methods. We have not in mind here so much the uncertainty often present in the diagnosis of a positive Marchi degeneration, but rather two other objections: 1, the Marchi method does not and can not reveal the actual site of ending of the degenerating myelinated axons, for the last millimeters of these have no myelin sheaths; 2, this method cannot help out in the case of non-myelinated neurones. Both objections hold also for the Weigert method, which, furthermore, because it provides a "negative" picture of the degeneration, permits a diagnosis only if the degeneration has occurred in a fairly great number of axons gathered in more or less compact bundles. Similarly the diagnosis of retrograde cell degeneration can only be made if the cells which have atrophied or disappeared were numerous and formed a fairly compact nuclear mass.

For the last four years we have used in this laboratory a combination of physiological methods which has shown itself admirably suitable for the study of problems of "functional organization" in the CNS (1, 2, 3, 4, 5, 6, 7), namely, the local strychninization of a mass of gray matter in combination with recording of the electrical activity of some other gray matter in the CNS. Strychnine solution applied to a few square millimeters of superficial gray matter, i.e., local strychninization of this mass, results in a typical change in its electrical activity, namely, the appearance of large, rapid voltage fluctuations, "strychnine-spikes." When the local

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strychninization has been performed somewhere in the sensorimotor cortex these spikes do not remain restricted to the small area strychninized but are also found in other specific areas of this cortex. The distribution of the strychnine-spikes is specific for each strychninized area. The observations that local strychninization of an area *a* (for instance area 6) produces spikes in the electrogram of some other area *b* (for instance area 4), whereas local strychninization of area *b* does *not* produce spikes in the electrogram of area *a* show that this combination of methods reveals *directed* functional relations and, therefore, directed anatomical relations. To correlate these findings with the neuronal structure of the cortex two suppositions are possible: 1, that strychnine acts on the axonal terminations and produces its remote effects by antidromic "firing" of the cell bodies of the neurones whose axonal endings are strychninized; 2, that strychnine acts on the cell bodies (and [or] the synapses incrusting these) and produces its remote effects in the direction of the normal conduction in the neurones. That the first of these suppositions is false and the second true follows from the following observations. Local strychninization of the dorsal horn of a spinal segment invariably evokes symptoms of sensory excitation (paresthesiae, hyperesthesia, hyperalgesia) in the corresponding "strychnine-segment zone" and only in this zone (8). The axonal terminations converging upon the perikarya of the posterior horns of a spinal segment are the endings of neurones originating in many other spinal segments and supraspinal levels. If the strychninization of these axonal terminations "fired" antidromically the cell bodies of the neurones to which they belong, it is impossible to conceive how the sensory symptoms could be projected by the animal into the periphery, into the strychnine-segment zone of the locally strychninized spinal segment and in this zone only. On this supposition the symptoms of sensory excitation should be absent altogether or present in a much larger area of the body, namely, in all portions subserved sensorially by the neurones "fired" antidromically. A second observation which precludes the first supposition is that local strychninization of the posterior horn of a spinal segment does not produce strychnine-spikes in the corresponding posterior root (unpublished experiments). The only alternative left is, therefore, the second supposition mentioned, namely, that the strychnine acts on the cell bodies (and [or] the synapses incrusting them) and produces its remote effects in the direction of the normal, i.e., forward, conduction in the reflex arc.

This view is strengthened by the following observations. Local strychninization of a specific area of the sensorimotor cortex, to wit, areas L. or A.4-s, produces strychnine-spikes in the electrogram of the nucleus caudatus, whereas strychninization of this nucleus does not "fire" the cortex (6). Several neuroanatomists are inclined to admit the existence of cortico-caudate neurones, all deny the existence of caudato-cortical neurones.

Local strychninization of the sensorimotor cortex "fires" the sensory thalamic nuclei and local strychninization of these nuclei "fires" the sensorimotor cortex. Here, neuroanatomy recognizes both cortico-thalamic and thalamo-cortical neurones. All available evidence, therefore, points towards the thesis that strychnine acts on the perikarya (and [or] the synaptic endings incrusting them), causes these structures to discharge impulses and, by axonal propagation of these impulses, produces strychnine-spikes in those remote, related, portions of gray matter where axons originating in strychninized nerve cells or collaterals of such axons end. If this thesis is accepted, local strychninization with recording of the electrical activity in the course of an acute experiment of a few minutes in the living (narcotized) animal gives the origin and termination of neurones. Thus one obtains physiological delimitation of neurones in the CNS.

This thesis has been verified again in a new series of experiments on the sensory systems between the nuclei gracilis (Goll) and cuneatus (Burdach) and the sensory cortex. For a clear understanding it is necessary to say a few words about the make-up of these systems. In the nuclei gracilis and cuneatus originate the fibers of the medial fillet (lemniscus medialis) which end in the sensory nuclei of the optic thalamus, where begin the tertiary sensory neurones that end in the sensory cortex. Present-day anatomy asserts that all fibers of the medial fillet have a synaptic interruption in the thalamus; a few of the older neuroanatomists admitted also the existence of an uninterrupted path from the nuclei gracilis and cuneatus to the sensory cortex, i.e., of a direct cortical fillet. In the descending direction, while neuroanatomy recognizes the existence of cortico-thalamic neurones, there is nothing to suggest the existence of neurones descending from the sensory cortex to the nuclei gracilis and cuneatus.

If there is no such descending system and if there is a direct cortical fillet, one obviously has an opportunity for verification of our thesis. For then strychninization of the arm and leg regions of the sensory cortex should not produce strychnine-spikes in the electrogram of the nuclei gracilis and cuneatus, whereas strychninization of these nuclei should result in strychnine-spikes in the electrocorticograms of the leg and arm subdivisions of the sensory cortex.

When put to the test in experimentation both predictions are verified. Even strychninization of the cortex of almost the entire postcentral gyrus (leg and arm) produces no change in the electrograms of the Goll and Burdach nuclei; strychninization of these nuclei produces definite strychnine-spikes in the electrograms of the corresponding subdivisions (leg and arm) of the sensorimotor cortex.

In explanation of the appearance of these strychnine-spikes two possibilities present themselves: 1, that these spikes result from "firing" of cells in the nuclei gracilis and cuneatus whose axons end directly in the

sensory cortex; 2, that they are the expression of disturbances propagated to the cortex through synaptic relays in the thalamus. Transsynaptic conduction of strychnine-spikes under the conditions of this experiment is improbable for reasons which will be discussed later. But quite apart from the possibility of transsynaptic conduction of the strychnine-spikes, the problem at hand can be settled by removing the thalamus without injury to the internal capsule—through which the neurones of the direct cortical fillet, if present, must ascend—and then repeating the experiment.

Therefore, in a monkey (macaca mulatta) under Dial-narcosis, one hemisphere was completely removed and the thalamus of the remaining hemisphere scooped out. Then a pair of stigmatic electrodes was placed on the sensory cortex in area arm 4 (A.4). Local strychninization of the contralateral nucleus cuneatus resulted in the appearance of definite strychnine-spikes in the electrocorticogram of A.4.

This cortex showed, naturally, very little spontaneous electrical activity due to the extirpation of its thalamus, by which procedure the cortico-thalamo-cortical circuits, necessary for the maintenance of the normal electrocorticogram, were destroyed. In this experiment the nucleus cuneatus and the arm region of the sensory cortex were preferred to the nucleus gracilis and the leg region because the sensory arm nuclei of the thalamus lie medial to the leg nuclei (9); thus total extirpation of the arm nuclei without injury to the internal capsule can be performed with greater certainty.

Records 2 and 3 of figure 1 show the presence of strychnine-spikes in the electrogram of the sensory (arm) cortex 17 and 25 minutes respectively after strychninization of the contralateral nucleus cuneatus; record 4 shows that these spikes have disappeared again 35 minutes after this strychninization. Figure 2 shows the brain from which these records were obtained. This experiment demonstrates that local strychninization of the nucleus cuneatus still produces strychnine-spikes in the sensory arm cortex, even in the acute experiment, after extirpation of the thalamus.

In two other similar experiments no such spikes appeared and the autopsies revealed that in these monkeys not only was the thalamus removed, but in one case the posterior limb of the internal capsule interrupted, and in the other the medial fillet just below the level of the thalamus. From this evidence it follows that in the nucleus cuneatus originate neurones, whose axons run through the internal capsule directly to the sensory (arm) cortex. In other words, there exists a direct cortical fillet.

It almost goes without saying that when the thalamus is left intact, strychninization of the nuclei gracilis and cuneatus produces strychnine-spikes of the electrograms of the corresponding sensory thalamic nuclei (leg and arm nuclei), thus revealing the existence of neurones originating in the nuclei gracilis and cuneatus and terminating in the thalamus (secondary sensory neurones). The existence of the tertiary neurones of this

sensory system and their distribution in the sensory cortex has already been established, as mentioned above, by these physiological methods in previous experiments (5).

In these experiments on the relations of the nuclei gracilis and cuneatus and the thalamic nuclei, three electrodes, insulated except for their tips, intended for "triangulation" of the electrical activity of the thalamus were usually introduced into it. That is, the three electrodes (a, b and c) were

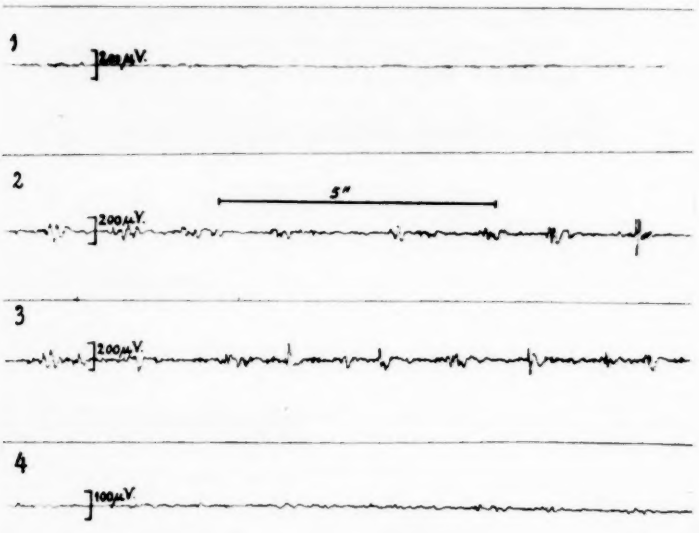

Fig. 1. Macaca mulatta. Dial-narcosis. Total extirpation of the *left* hemisphere and scooping out of the *right* thalamus. Four electrograms from the *right* precentral arm cortex (A.4) before (record 1) and after (records 2, 3 and 4) local strychninization of the *left* nucleus cuneatus. Record 2 taken 17 minutes after this strychninization, record 3, 8 minutes later, record 4 again 10 minutes later (35 minutes after the strychninization) when strychnine-spikes in records 2 and 3 have disappeared. Note that the electrical activity of the cortex is very small, because of the interruption of the cortico-thalamo-cortical circuits by the extirpation of the thalamus.

linked up with three oscillographs in the following manner: electrodes a and b to oscillograph 1, electrodes b and c to oscillograph 2 and electrodes c and a to oscillograph 3. This arrangement gives perhaps in the simplest way clear-cut evidence where a disturbance evidenced in the electrograms originates. If originating at or in the neighbourhood of electrode a it will appear in oscillograms 1 and 3; if the disturbance starts at or near electrode b it will manifest itself in oscillograms 1 and 2; if the disturbance originates at or near electrode c it will appear in oscillograms 2 and 3.

In one of these experiments strychnine-spikes appeared in the electrograms referable to electrodes a and c, but not in those to electrode b. See figure 3.

At autopsy it was found that, because of forgetting in this particular experiment to orient the head in space, the electrodes were not introduced into the thalamus but that electrodes a and c were in the globus pallidus whereas b was in the putamen!

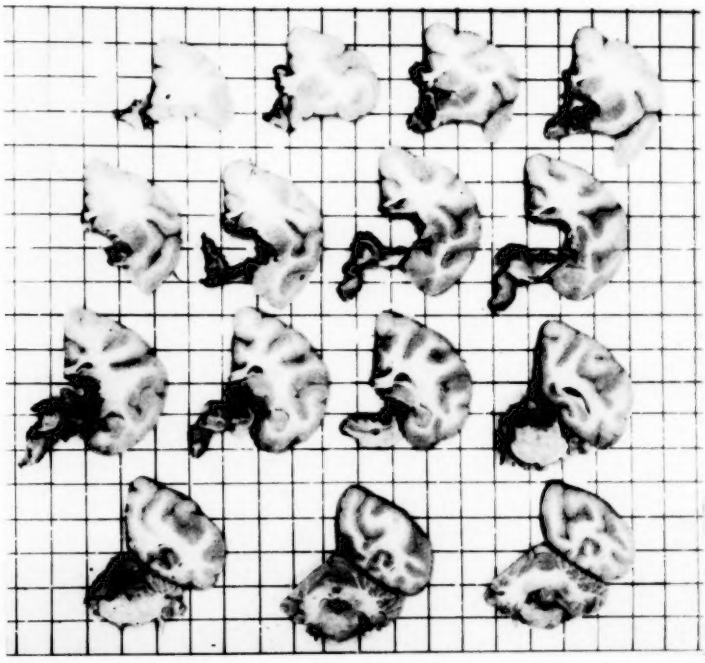

Fig. 2 shows brain of experiment of figure 1. Total extirpation of left hemisphere and right thalamus. Squares of background represent square centimeters.

In records II and III of figure 3 it will be seen that strychnine-spikes are present simultaneously in electrograms 2 and 3 and in 1 and 3 respectively, indicating that leads a and c (which were in the pallidum) were "fired." The absence of a simultaneous disturbance in electrograms 1 and 2 denotes that electrode c, lying in the putamen, was not fired.

Electrode a was so close to the fibers of the internal capsule, although definitely in the gray matter of the pallidum, that it might be thought that the spikes referable to this electrode might be spikes "passing by" in the fibers of the internal capsule,

namely, spikes in the fibers of the direct cortical fillet fired by the strychninization of the nuclei gracilis and cuneatus.

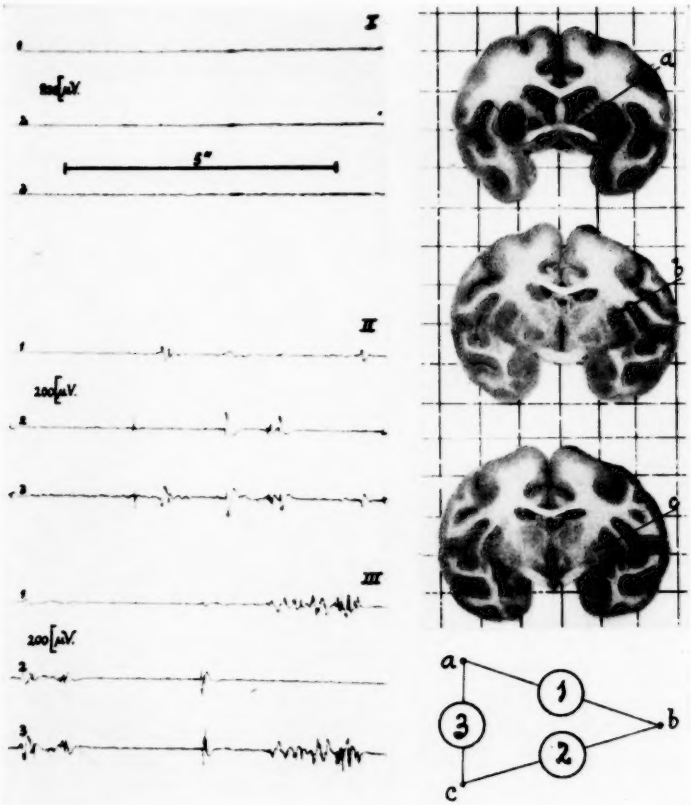

Fig. 3. Macaca mulatta. Dial-narcosis. Electrograms taken by the "triangulation" arrangement (see text) from pallidum and putamen. Position of electrodes and their "hook-up" at right of records. Record I (control) before, records II and III after local strychninization of contralateral nuclei of Goll and Burdach. Simultaneous disturbances in electrograms 1 and 3 denote "firing" at electrode *a* (in pallidum), such in electrograms 2 and 3 firing at electrode *c* (in pallidum). Absence of synchronous disturbances in electrograms 1 and 2 signifies absence of firing at electrode *b* (in putamen).

This possibility cannot be accepted, however, for electrode *c*, whose tip lay in the peripheral portion of the inner segment of the globus pallidus and it will be seen in figure 3 that the most typical strychnine-spikes appear in electrograms 2 and 3, referable to electrode *c*.

We were much surprised by this firing of the pallidum by the strychninization of the nuclei gracilis and cuneatus, indicating the existence of neurones originating in these nuclei and terminating in the pallidum, for we did not know of the existence of such neurones. But scrutiny of the literature revealed that Bechterew as far back as 1885 (see his Leitungsbahnen im Gehirn und Rückenmark, [A. Georgi, Leipzig], 1899, pp. 239–241) on the basis of myelogenetic studies indicated the existence of such a pathway and that Cajal in his Histologie du système nerveux ([Maloine, Paris], vol. 2, 1911 p., 511) describes collaterals arising from fibers of the medial fillet and ending in the globus pallidus.

One point remains for discussion, namely, the question of transsynaptic propagation of the strychnine-spikes. This whole series of experiments on strychninization of the nuclei gracilis and cuneatus and recording the electrical activity of the sensory cortex was started to investigate this very problem on the assumption that *all* secondary sensory neurones originating in these nuclei terminate in the thalamus. The unexpected physiological demonstration of neurones from these nuclei running directly up to the sensory cortex precluded the intended investigation in this particular system. All that can be said at present is that so long as one adheres to the procedure of *local* strychninization in the narcotized animal there is no direct evidence of such transsynaptic propagation. By local strychninization is meant either the strychninization of only a few square millimeters of superficial gray matter of the CNS (cortex, spinal cord, etc.) or the injection of a minute quantity of a strychnine solution (less than 1 cubic millimeter of a 1 to 3 per cent solution) into deep structures of the CNS (thalamus and other subcortical structures).

Such local strychninization when performed on the "motor" cortex of the (narcotized) macaque never results in twitches in the peripheral striped musculature. These only appear when the strychninization is much more extensive or often repeated without waiting for the effect of previous local strychninizations to wear off. Obviously such twitches must be the expression of transsynaptic disturbances, though it is still an open question whether these are synchronized enough to resemble typical strychnine-spikes.

Occasionally, after strychninizations which are not strictly local, one observes in the electrogram of gray matter of the CNS, which according to present-day neuroanatomy is only in transsynaptic connection with the strychninized gray, a small and relatively slow "bump". This, however, is not easily confused with the typical strychnine-spike.

Until evidence to the contrary is forthcoming we feel compelled to regard the appearance of strychnine-spikes in the electrogram of a gray mass of the CNS following local strychninization of another gray mass as evidence

of the strychninization of neurones originating in the mass strychninized and ending in or giving collaterals to the gray matter where the spikes appear. Therefore, this combination of physiological methods allows one in the course of an acute experiment of a few minutes' duration to delimit the origin and ending of neurones in the CNS, and thus to confirm anatomy where it is known, to settle mooted anatomical questions and even to discover unknown neuronal connections which have not been and cannot be investigated adequately by anatomical methods now known.

CONCLUSIONS

It is shown that the method of local strychninization of gray matter combined with the recording of the electrical activity of another mass of gray matter of the CNS permits one in the course of an acute experiment of a few minutes in the fully narcotized animal to decide whether in the strychninized gray mass originate neurones ending, directly or with axonal collaterals, in the gray matter whose electrical activity is recorded. If strychnine-spikes appear in this electrogram the answer is in the affirmative, if no spikes appear in the negative.

With these physiological methods the existence of a direct cortical fillet (from the nuclei cuneatus and gracilis to the sensory cortex) and the existence of neurones originating in the nuclei cuneatus and gracilis and ending in the globus pallidus has been demonstrated.

This combination of physiological methods, therefore, permits the delimitation of neurones in the central nervous system.

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THE GONADOTROPIC CONTENT OF THE FEMALE RAT HYPOPHYSIS IN EXPERIMENTAL PRECOCIOUS PUBERTY[1, 2]

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In a recent publication (1) it was shown that the gonadotropic content of the female rat hypophysis underwent a marked decrease concomitant with the onset of puberty. This was interpreted to mean that the progressively increasing amount of estrogen secreted by the maturing ovary caused an increasingly rapid release of hormone from the pituitary. The purpose of the present investigation was to make a similar study of artificially induced precocious puberty.

PRELIMINARY EXPERIMENTS. Hypophyses were removed from immature female rats which had been employed in the assay of pituitary gonadotropic hormones. These rats (designated as primary test animals) had received, twice daily for three days, varying doses of suspensions made from hypophyses of normal immature and mature and castrated female rats. All degrees of increase in uterine and ovarian weights resulted. There were from 8 to 12 rats in each group. At autopsy, pituitaries from all rats of each group were pooled and suspended in water by a method previously described (1, 2), and assayed on 22-day old female rats weighing 35 to 40 grams (designated as secondary test animals). The resulting uterine weights were used as an index of the gonadotropic activity. The number of uterine units per average hypophysis was calculated from the same dosage-response curve as was used in the previous study (1). The average pituitary from untreated normal 25-day old female rats contains 1.8 uterine units.

Table 1 summarizes the results of these preliminary experiments. In the left-hand columns are given the mean uterine and ovarian weights of the primary test animals. The right-hand columns present similar data from the assays of hypophyses taken from the primary test rats. In some

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[2] Presented before the 51st Annual Meeting of the American Physiological Society, Toronto, Canada, April 28, 1939.

TABLE 1

Results of preliminary experiments

PRIMARY TEST ANIMALS					SECONDARY TEST ANIMALS					
Source of gonadotropic hormone*	Number of rats	Total dose	Mean uterine weight	Mean ovary weight	Number of rats	Total dose	Mean uterine weight ± S.E.	Mean ovary weight ± S.E.	Uterine units per gland	Per cent change from normal
		mgm.	*mgm.*	*mgm.*		*gland equiv.*				
21-day	12	2.0	113.4	33.8	8	½	26.1± 1.8	11.3±1.0	1.1	−40
normal					7	1	63.4± 6.0	15.1±0.8		
16-week	12	21.3	76.1	13.7	8	½	26.8± 2.4	11.7±0.6	1.5	−17
normal					7	1	112.2± 4.1	20.0±1.1		
5-day	12	5.6	44.7	11.1	8	½	40.0± 4.0	12.8±0.5	1.8	0
castrate					8	¾	94.0± 5.0	16.3±0.9		
	12	11.2	102.5	16.0	10	¾	45.8± 5.1	13.5±0.7	1.2	−33
10-day	12	1.5	31.4	11.5	9	¾	89.8± 6.5	16.5±1.3	2.0	+11
castrate	12	7.0	101.4	15.2	10	¾	56.8±10.3	11.8±0.6	1.4	−23
30-day	12	1.0	62.7	12.8	8	½	52.9± 6.1	11.2±0.7	2.0	+11
castrate					8	¾	100.5±10.0	18.0±0.8		
60-day	12	0.75	93.5	13.2	10	¾	92.5± 7.0	17.9±1.1	2.0	+11
castrate	12	1.5	117.4	17.3	10	¾	84.4± 7.9	16.6±1.1	1.9	+6
120-day	8	1.0	92.6	13.8	8	¾	84.3± 8.8	15.1±1.2	1.9	+6
castrate	9	3.0	110.4	25.6	8	1	57.6± 8.6	12.4±1.0	1.1	−40
	12	8.0	86.1	78.9	7	1½	109.2± 6.5	17.9±1.9	1.1	−40
Assay of 25-day normal females**					12	¼	22.0± 0.7	11.3±0.4	1.8	
					12	½	45.9± 4.8	12.0±0.4		
					12	¾	76.7± 8.3	15.6±0.9		
					12	1	121.9± 4.0	22.3±1.3		

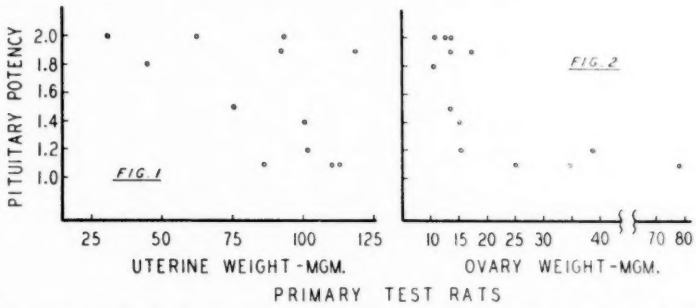
S.E. = standard error of the mean = $\frac{\sigma}{\sqrt{N}}$.

* Female rat pituitary was the source of hormone in all cases. All castrates were 24 weeks old at time of sacrifice.

** Data taken from previous publication (1). These values formed the basis for the dosage-response curve used to estimate the potency of the hypophyses for all the groups reported in the present paper. Uterine weights of the secondary test animals may be compared directly with these figures.

instances, two dose levels were employed for the estimation of the pituitary potency. The calculated number of units per gland are given in next to the last column. In figures 1 and 2 the potency of the pituitaries is plotted

against the mean uterine and ovarian weights of the primary test animals. A distinct inverse correlation is seen between the degree of sexual development and the hypophyseal gonadotropic content. In all cases in which the ovarian weight exceeded 20 mgm. the potency was reduced 33 to 40 per cent below that of normal 25-day old controls. This reduction is statistically quite significant. The slight increases (1.9, 2.0 units per gland) are not significant. There seems to be a definite limit below which, in spite of further increase in ovary weight, the potency does not fall. This may mean either *a*, that no more than about 40 per cent of the contained gonadotropic complex is available for release during the three-day period of stimulated ovarian activity, or *b*, that the rates of increased production and release reach a balance at such level that the content at the time of autopsy is about 40 per cent less than normal.



Figs. 1 and 2. Preliminary experiment. Showing the inverse correlation between mean uterine and ovarian weights of the primary test rats and the average pituitary gonadotropic potency, expressed in terms of uterine units.

Primary test animals were injected at two dose levels in the cases of three of the gonadotropic preparations. The hypophyses from these groups were, in turn, assayed on secondary test rats, all of which received the same total dose of $\frac{3}{4}$ gland-equivalent. The data from these special groups are given briefly in table 2, arranged to emphasize the reciprocal relationship between the degree of uterine stimulation and the decrease in hypophyseal potency. Where the uterine weight was low in the primary rats, the hypophyses from these animals elicited a high uterine response, and vice versa. When the difference in the primary uterine weights was not large, as in the third experiment, the difference between the two groups of secondary uterine weights is not significant. The first experiment is especially interesting. The uterine weights in the primary groups were 44.7 and 102.5 mgm., whereas the responses elicited in the secondary animals by $\frac{3}{4}$ gland doses of pituitary from the primary groups were the reverse, namely, 94.0 and 45.8 mgm., respectively. When the hypophyses

from the secondary rats were assayed, in turn, on tertiary test animals, the uterine weights were again reversed. The respective differences are statistically significant. Clearly, the hypophyseal gonadotropic potency of sexually precocious rats is inversely proportional to the degree of uterine development.

FINAL EXPERIMENT. Because of the considerable variation in uterine weights encountered in the above described experiments, it was thought essential to conduct a more carefully controlled study. For this purpose 12 four-female litters were separated into four groups of 12 rats, with each litter represented in all groups. The rats of two groups were ovariectomized on the 21st day of life. Beginning on the next day, the rats of

TABLE 2

Data from special groups of the preliminary experiments

PRIMARY TEST ANIMALS				SECONDARY TEST ANIMALS				TERTIARY TEST ANIMALS			
Source of gonadotropic hormone	Number of rats	Total dose	Mean uterine weight	Number of rats	Total dose	Mean uterine weight ± S.E.	P.* of diff.	Number of rats	Total dose	Mean uterine weight ± S.E.	P.* of diff.
		mgm.	*mgm.*		*gland equiv.*	*mgm.*			*gland equiv.*	*mgm.*	
5-day	12	5.6	44.7	8	¾	94.0± 5.0	<0.01	8	¾	45.4± 3.7	0.04
castrate	12	11.2	102.5	10	¾	45.8± 5.1		8	¾	73.1±12.8	
10-day	12	1.5	31.4	9	¾	89.8± 6.5	<0.01				
castrate	12	7.0	101.4	10	¾	56.8±10.3					
60-day	12	0.75	93.5	10	¾	92.5± 7.0	0.49				
castrate	12	1.5	117.4	10	¾	84.4± 7.9					

* P. is the probability that the difference is due to chance, and therefore not a true difference.

one normal and one castrated group were injected twice daily for three days, and all groups were autopsied on the 25th day of life. All the injected rats received the same dose of an immature rat pituitary suspension, which was calculated to produce ovaries in the normal rats, weighing about 35 mgm. At autopsy, each individual hypophysis was suspended in 4 cc. of sterile distilled water. The individual suspensions were assayed on 12 four-female litters of 22-day old rats, each test animal receiving a total of 3 cc. in six injections, i.e., a dose of ¾ gland-equivalent. These secondary test animals were autopsied at 25 days of age.

The results are summarized in table 3. It is seen that the pituitaries of the normal non-injected and both castrated groups are equally potent and similar to the normal controls of the preliminary studies (table 1).

On the other hand, the hypophyses from the normal injected rats show a distinct decrease in potency. Table 4 gives the significance of the differences of uterine and ovarian weights among the four groups of secondary test animals. The injected normal group differs very significantly from the other three. The individual uterine and ovarian weights are shown

TABLE 3

Results of final experiment

PRIMARY TEST ANIMALS				SECONDARY TEST ANIMALS				
Number of rats	Treatment	Mean uterine weight ± S.E.	Mean ovary weight ± S.E.	Number of rats	Total dose	Mean uterine weight ± S.E.	Mean ovary weight ± S.E.	Uterine units per gland
		mgm.	*mgm.*		*gland equiv.*	*mgm.*	*mgm.*	
12	Normal—not injected	22.3±1.1	13.2±0.6	12	¾	85.4± 8.3	20.4±2.5	1.9
12	Normal—injected	123.4±5.0	38.9±3.1	12	¾	44.2± 6.4	12.3±0.6	1.2
11*	Castrate—not injected	19.7±0.6		11	¾	78.6±10.6	18.0±1.9	1.8
12	Castrate—injected	19.7±0.5		12	¾	83.5± 9.7	17.0±1.5	1.9

* One rat died during ovariectomy.

The injected primary rats received an immature female rat pituitary suspension twice daily for three days and were autopsied on the fourth day.

TABLE 4

Significance of the differences of mean uterine and ovary weights from the secondary test animals in final experiment

GROUPS COMPARED	UTERINE WEIGHT			OVARY WEIGHT		
	Diff.	$\frac{\text{Diff.}}{\sigma_{\text{diff.}}}$	P. of diff.	Diff.	$\frac{\text{Diff.}}{\sigma_{\text{diff.}}}$	P. of diff.
	mgm.			*mgm.*		
Normal injected—normal not injected	41.2	3.92	<0.01	8.1	3.17	<0.01
Normal injected—castrate not injected	34.4	2.77	<0.01	5.7	2.86	<0.01
Normal injected—castrate injected	39.3	3.38	<0.01	4.7	2.86	<0.01
Normal not injected—castrate not injected	6.8	0.50	0.61	2.4	0.77	0.44
Normal not injected—castrate injected	1.9	0.15	0.88	3.4	1.17	0.24
Castrate not injected—castrate injected	4.9	0.34	0.73	1.0	0.41	0.68

in figures 3 and 4. In spite of the wide variation, the differences are clear. A large part of this variability can be attributed to differences in the reactivity of the secondary test animals rather than to differences in the true potency of the individual primary pituitaries, since the variation in uterine and ovarian response is almost as great when pooled pituitary suspension is injected as when individual glands are assayed. This is

brought out by comparison of the standard errors in the preliminary and final experiments.

Discussion. The results of this investigation are clear. In precocious puberty, as in normal puberty, the gonadotropic content of the hypophysis comes to be inversely proportional to the degree of sexual development. Inasmuch as the methods used do not measure the individual hormones of the gonadotropic complex but merely the sum total of their actions, conclusions cannot be drawn with certainty. However, in an experiment which parallels ours, Hohlweg (3) found that a week after administering a single large dose of estrogen to immature female rats, the gonadotropic content of the hypophysis was reduced considerably below normal. At

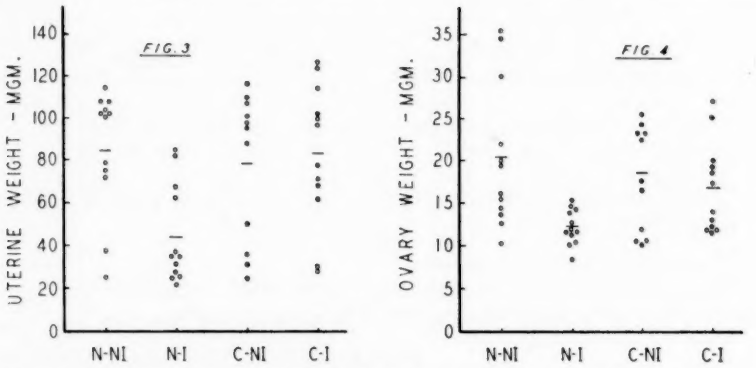


Fig. 3. Final experiment. Individual uterine responses of secondary test rats receiving $\frac{3}{4}$ gland-equivalent of pituitary suspension from normal non-injected 25-day-old female rats (N-NI), from normal females of the same age which had been injected with gonadotropic hormone (N-I), from castrated females of the same age (C-NI), and from castrated females of the same age (C-I) which had been injected with the same amount of gonadotropic hormone as the N-I group. All pituitaries were assayed individually.

Fig. 4. Final experiment. Individual ovarian responses of the same rats.

the same time the ovaries showed corpus luteum formation, indicating the release presumably of luteinizing hormone. It seems possible that a similar process occurs under the experimental conditions described above. That is, the injected gonadotropic substance stimulates the ovaries to produce estrogen which in turn possibly causes the hypophysis to release gonadotropic material (mostly LH ?) into the circulation. On the basis of cytological observations, A. E. Severinghaus (4) concluded that estrogen under certain conditions causes the pituitary to produce and release increased quantities of gonadotropic hormone. It is difficult to reconcile this concept of stimulation of the hypophysis by estrogen with the older idea of inhibition. It may be possible, however, that estrogen may both

stimulate and depress, as was postulated by Fevold, Hisaw and Greep (5), whose studies have led to the conclusion that estrogen stimulates the production and release of LH and inhibits the output of FSH.

Gonadal development, chiefly in the direction of luteinization, has been found to result from estrogen administration by a number of investigators since the initial discoveries of Hohlweg (6, 7, 8, 9, 10, 11, 12, 13). Similar gonadotropic effects have recently been obtained with androgens (14, 15, 16). Evidence favoring the above concept of estrogen stimulation of the anterior lobe has been reported by Victor and Andersen (17, 18), who found an increased oxygen consumption in hypophyses under estrogen influence.

In a previous report (1) it was shown that the immature female rat hypophysis contains relatively and absolutely more gonad-stimulating substance than is found at any other age period, excepting senility. In such infantile animals, the high pituitary content and the concurrent lack of sexual development indicates a very slow rate of release. When the hypophysis is exposed to estrogen influence, whether from stimulated ovaries at the time of puberty or from injection, the rates of production and release are possibly stimulated. The content of the hypophysis at any given time, therefore, only measures the level at which these two processes are balanced.

The obvious implication which follows from the above discussion is that when intact immature females are employed in gonadotropic assays, the test animal's own hypophysis probably contributes to the ovarian reaction, augmenting the effect of the injected material. The larger the dose, the greater the participation, up to a limit, on the part of the pituitary. The work of Fevold, Hisaw and Greep (19) and Tyndale, Levin and Smith (20) offers convincing evidence in favor of this view.

SUMMARY

A study has been made of the gonadotropic content of pituitaries from immature female rats in which precocious puberty had been induced by gonad-stimulating hormone. The hypophyseal potency was found to be inversely proportional to the degree of sexual development. In all cases in which the weight of the ovaries exceeded 20 mgm., the potency was reduced 33 to 40 per cent below control level. In the light of recent reports, these findings are interpreted to mean 1, that under these experimental conditions estrogen from the stimulated ovaries probably increases the rate of production and release of gonadotropic hormone from the hypophysis, and 2, that the decreased potency indicates that the balance between these two processes is adjusted at a lower level than in the normal immature rat.

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THE EFFECT OF ANOXIA ON GASTRIC SECRETION FROM PAVLOV AND HEIDENHAIN POUCH DOGS

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Bayeux in 1911 showed that the volume of gastric juice diminished and that total acid was not affected at an altitude of 14,000 feet. Delrue in 1934 found that the gastric secretion of dogs taken to an altitude of 8,000 feet showed a decrease of pH and total acid. Hellebrandt, Brogdon and Hoopes in 1935, working on humans and using a rebreathing apparatus, reported that anoxemia of the pre-coma type caused no appreciable decrease in gastric acidity. In 1937 Sleeth and Van Liere, working on the response of the stomach to water in barbitalized dogs, found that the acidity and chlorides were only definitely decreased after the animals had been subjected to a partial pressure of oxygen of 63 mm. Hg, corresponding to an altitude of 28,000 feet.

It is quite apparent from the above data that further well controlled work is needed on the effect of anoxemia on the quantity and quality of gastric secretion.

METHODS. To insure the collection of pure gastric juice, dogs with gastric pouches were used. Four dogs with the Pavlov pouch and three dogs with the Heidenhain pouch comprised the experimental animals. A large respiration chamber of the type described by Van Liere was used to produce the anoxia. The accumulation of CO_2 was prevented by adequate ventilation. It also was arranged so that the temperature within the chamber could be controlled. The animals were maintained on a standard diet of white bread, ground beef heart, and whole milk.

All the control and experimental work was done in the respiration chamber. The animals were placed in their stocks in the chamber for an hour in order to obtain the basal secretion. They were then fed a standard meal of 250 grams of ground beef heart. This was prepared by bringing it to a boil in 250 cc. of tap water and allowing it to cool. As a rule the animals ate this meal with relish. If they refused to eat all the meal, they were not used on that day.

For the control experiments the valves of the respiratory chamber were left open and the air pump was allowed to run so as to simulate as nearly as possible the experimental conditions. Samples of gastric secretion were collected every half-hour for five hours after feeding. The samples were

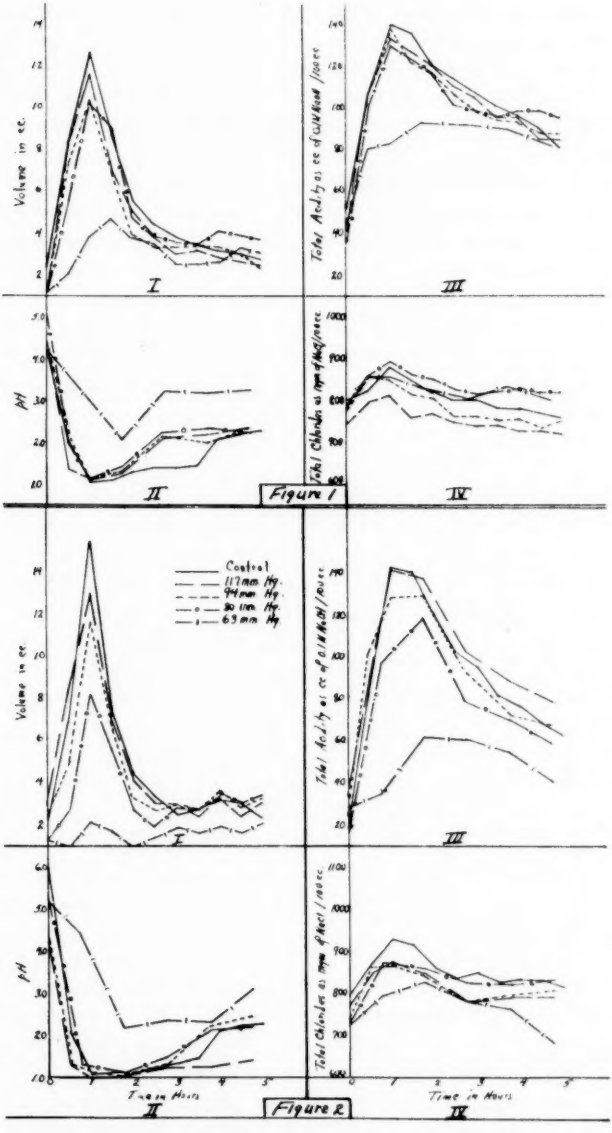
analyzed for concentration of total acids per 100 cc. using 0.01 N NaOH with phenolphthalein as an indicator; for total chlorides using a Van Slyke modification of the Volhard method; and for pH using a Beckman meter equipped with glass electrodes.

The anoxia experiments were performed using partial pressure of oxygen of 117, 94, 80 and 63 mm. Hg, corresponding to altitudes of approximately 8,000, 14,000, 18,000 and 24,000 feet respectively. The procedure was the same as for the control experiments. Care was taken to allow sufficient time to elapse between anoxia experiments so that acclimatization was kept at a minimum.

When the experimental work was begun, an investigator remained in the chamber with the animals throughout the entire experiment to remove half hour samples. The severe degrees of anoxia, however, made the investigators ill, so after a few experiments had been conducted in this manner, the investigators remained outside the chamber allowing the dogs to return to atmospheric pressure every hour so that samples could be removed. Since it was found that data collected in this manner compared favorably with data collected every half hour with an investigator in the tank, the remainder of the experiments were completed collecting the hourly samples. The animals withstood the anoxia very well and showed no outward signs of distress during the experiments.

Results. Figure 1 represents the averages of all the experimental results on the Pavlov group. Each animal follows the average curves, so that it presents a true average. Graph I indicates a steady fall in the volume of gastric juice secreted, as the anoxia becomes more severe, but the fall is not statistically significant until a partial pressure of oxygen of 80 mm. Hg is reached. At a partial pressure of oxygen of 63 mm. Hg the decrease in volume below the normal is very marked, indicating that the threshold in dogs for the diminution of volume is approximately 80 mm. Hg. Graph II illustrates that pH is not noticeably affected until an anoxia of 63 mm. Hg is produced at which point it is statistically significant. Graph III shows that a steady decline of total acidity is produced by anoxia. By statistical analysis the decline is found to be significant at a partial pressure of oxygen of 80 mm. Hg. Graph IV demonstrates that total chlorides are not affected by anoxia; this is borne out by statistical analysis.

Figure 2 comprises the averages of all the experiments performed with the Heidenhain group. Each animal followed the average curve. Graph I, in this group, indicates a sharper decrease in volume which becomes statistically significant at a partial pressure of oxygen of 94 mm. Hg. Graph II shows that pH rises as anoxia progresses and there is a statistically significant difference at 80 mm. Hg. Graph III indicates that total acidity is apparently affected at a partial pressure of oxygen of 94 mm. Hg. However, statistical analysis shows that the difference is not significant



until a partial pressure of 63 mm. Hg is reached. Graph IV indicates that anoxia causes a diminution of total chlorides. Statistical analysis shows the difference to be significant at 94 mm. Hg.

Discussion. Since the volume of gastric juice is distinctly diminished

in both groups, it is evident that appreciable degrees of anoxia depress the activity of the secretory cells of the stomach. The results clearly show, however, that the Heidenhain group was affected by less severe degrees of anoxia than the Pavlov group. This indicates that the vagus is concerned. It is well known that anoxia is capable of stimulating the medullary centers.

As the results indicate, the total acid secretion diminishes at a partial pressure of oxygen of 80 mm. Hg while the pH is not affected until a more severe degree of anoxia is reached (63 mm. Hg). It is suggested that the decrease in total acidity is accompanied by a decrease in buffer secretion, since the pH does not directly follow total acid secretion. The chlorides also do not follow the total acidity curve, being unchanged even at the most severe degrees of anoxia used, namely, a partial pressure of oxygen of 63 mm. Hg. For this reason it is evident that chlorides are being secreted in some form other than HCl, possibly as NaCl.

In the Heidenhain group the data, with the exception of volume, is the reverse from that of the Pavlov group. In this case the rise in pH beginning at a partial pressure of oxygen of 80 mm. Hg is not accompanied by a corresponding fall of the total acidity as occurs in the control experiments. Although the statistical analysis indicates that total acidity does not decrease significantly until a partial pressure of oxygen of 63 mm. Hg is reached, yet examination of graph III, figure 2, shows that the trend is definitely downward at even as high as 94 mm. Hg. These differences between the Pavlov and Heidenhain groups are most likely to be due to the difference in the innervation of the pouches since this is the only fundamental factor wherein the groups differ.

The data show that a diminution in quantity and quality of gastric secretion definitely occurs long before the pre-coma state of anoxia is reached. These findings do not coincide with those of Hellebrandt, Brogdon and Hoopes. Their work was done on fasting individuals given a test meal of gruel or alcohol to induce secretion. A rebreathing apparatus was used to induce the anoxia. Their results, therefore, can hardly be compared to those reported in this paper.

The work reported here agrees with that of Bayeux, who found a decrease in volume and no change in total acidity at 14,000 feet. It does not, however, corroborate the findings of Delrue, who reported a decrease in titrable gastric acidity and in pH at 8,000 feet. Both workers transported their dogs from their laboratories to others at higher altitudes. The dogs, then, were not only subjected to transportation, but also to an entirely different environment and presumably to a change in temperature. Any of these factors conceivably could affect gastric secretion.

The general level at which the diminution of quality and quantity of gastric juice occurs (80 mm. Hg) reported in this work compares favorably with what Barcroft termed the critical level of anoxemia.

Apperly and Cary found that at certain critical levels of anemic anoxia induced by hemorrhage, gastric acidity was reduced. Alvarez and Vanzant reported that a fall in hemoglobin, such as is caused by anemia, to 75 per cent was followed by a decrease in gastric acidity. It is of interest to note, then, that both the anoxic type of anoxia and the anemic type of anoxia produce a decrease in gastric acidity. It is likely that all types of anoxia may cause a diminution in gastric acidity, although we are not aware of any work reported on the effect of the stagnant type or the histotoxic type of anoxia on gastric acidity.

SUMMARY

Four dogs with the Pavlov pouch and three dogs with the Heidenhain pouch were subjected to partial pressures of oxygen of 117, 94, 80 and 63 mm. Hg, corresponding to altitudes of approximately 8,000, 14,000, 18,000 and 24,000 feet respectively.

There were differences in the two groups in their response to anoxia; the pH did not change in the Pavlov group until a partial pressure of oxygen of 63 mm. Hg was reached, whereas the Heidenhain group was affected at a less severe degree of anoxia (80 mm. Hg). The data obtained for total acids are exactly the reverse to the data obtained for pH. Total chlorides in the Pavlov group were not affected by the degrees of anoxia used in this work, but were decreased in the Heidenhain group as early as at a partial pressure of oxygen of 94 mm. Hg. In general, then, the Heidenhain group is affected by less severe degrees of anoxia than is the Pavlov group.

Anoxia caused a decrease in the volume of gastric juice secreted from the pouches of all the dogs; the more severe the anoxia, the greater the decrease of gastric secretion. Anoxia, however, affected the volume of the gastric juice of the Heidenhain group more than it did the Pavlov group.

The authors wish to give their best thanks to Dr. C. B. Pride and Dr. D. W. Northup for preparing the Pavlov and Heidenhain pouch dogs and to Paul E. Vaughan for valuable assistance in conducting experiments on the Pavlov pouch dogs.

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THE SPECIFIC DYNAMIC ACTION OF PROTEIN IN SYMPATHECTOMIZED CATS

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Among the various theories offered to explain specific dynamic action (s.d.a.) is the relatively recent one that the phenomenon involves the sympathetic nervous system. In 1923 Abelin reported that after repeated administration of substances which increase the irritability of the sympathetic (e.g., thyroid substance, tyramin, phenylethylamin, adrenaline) both the basal metabolic rate (b.m.r.) and, still more, the s.d.a. of meat were increased. The close relation between excitation of the sympathetic system, the height of the b.m.r. and the degree of s.d.a. of protein was confirmed by noting that after stopping the excitatory drugs the b.m.r. and the s.d.a. of protein gradually returned to their former value. In 1924 Liebesny, on the basis of experimental and clinical data, proposed a theory that "the amino-acid stimulus following the ingestion of protein, at least in some cases, acts first on the autonomic system and then on endocrine glands." Bahn (1926) suggested that the intensity of the s.d.a. of proteins was determined by the tonus of the autonomic nervous system. He assumed that various hormones change the s.d.a. indirectly by influencing the b.m.r.

In 1932, Noyons pointed out that his pupil Lips had found that the s.d.a. of protein is suppressed or delayed by gynergen (tartrate of ergotamine). "The most striking effects were obtained after bilateral splanchnotomy. In these circumstances the s.d.a. disappears entirely and even one year later the reaction does not occur. Unilateral splanchnotomy does not suppress the s.d.a." Noyons' communication was the starting point of new investigations by Rothschild and Wolfermann (1938). Repeating the experiments on dogs and cats under dial anesthesia and after injection of 933F, they observed that the s.d.a. was suppressed, and concluded that it was due to blocking of the sympathetic impulses. Also Schaeffer, Dontcheff and Le Breton (see Schaeffer and Le Breton, 1938) reported experiments on s.d.a. in rabbits, both after the administration of gynergen and after bilateral splanchnotomy. After gynergen they noted a "considerable diminution or even a complete suppression of the increased

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respiratory exchanges due to the ingestion of protein for the entire period during which the sympathicolytic action of the poison persists." And after bilateral splanchnotomy "the extra heat produced in these animals is considerably reduced in comparison to that obtained in normal ones—10 to 14 per cent instead of 30 per cent of the basal exchanges." Thus, in these latter conditions, the s.d.a. is not entirely suppressed. From these experiments Schaeffer and his collaborators concluded "that the cause of the extra-heat production, which characterizes the s.d.a., is a stimulation of the sympathetic nervous system by the amino-acids, as soon as their concentration in the organism reaches a certain value."

The foregoing results and interpretations are not in accord with those of Moore's experiments (1929) on cats subjected to sympathectomy, both partial (denervated heart) and complete. In all his cases, ingestion of lean beef or other protein induced regularly "an acceleration of the heart of surprising magnitude and duration" (25 to 50 per cent above the fasting level and persisting 15 to 20 hours). These results led Moore to the following interpretation: "In view of the close association which must exist between the rate of the pace-setting region and its metabolism, it has seemed reasonable to interpret the accelerations in terms of specific dynamic action."

The inconsistencies of the investigations mentioned above seemed to justify a new examination of the problem. The present research was undertaken in order to learn whether there is a relation between the s.d.a. of protein and the sympathetic nervous system.

METHOD. Cats were used, young adults in good physical condition, both the normal and the sympathectomized. Quiet animals were carefully selected in order to avoid anesthesia during the measurement of the respiratory exchange. Sympathectomy was performed as described by Cannon and his collaborators (1929). Both sympathetic trunks were removed from above the stellate ganglia to the level of the pelvic brim. They were removed in three stages: first, the left thoracic chain and the corresponding splanchnic; second, the abdominal chains, the right splanchnic and the ganglia of the solar plexus; third, the right thoracic chain.

The protein used for the determination of the s.d.a. was Witte's peptone (Merck). This peptone was preferable to lean beef, because it can be given always in definite quantity. The dose employed was 6 grams per kilogram of body weight. The peptone diluted in water at 30° was administered by a stomach tube. The s.d.a. under these conditions was regularly obtained. The b.m.r. was determined by a modified Benedict indirect, "closed circuit" method (cf. Fridericia, 1913; Friedgood, 1934), oxygen consumptions alone being used. The box containing the cat was in a water bath maintained at a constant temperature of 27.6° (the thermic neutrality of the cat being between 27.5 and 28°). Any slight movement

TABLE 1

Basal metabolic rates and maximal metabolic rates after administration of Witte's peptone, at various stages of sympathectomy and after complete sympathectomy

CAT NUMBER	DATE	WEIGHT	CALORIES PER SQ. M.		PERCENTAGE INCREASE B.M.R.	OPERATIONS (REMOVALS) 1939
			Basal	After peptone		
	1939	*kgm.*				
1	Mar. 1	2.20	25.41			*Feb.* 8: left thoracic chain and left splanchnic
	Mar. 4	2.19	26.34			
	Mar. 28	2.09	27.02	37.29	38.05	
2	Mar. 17	2.40	29.11	36.08	23.94	*Feb.* 13: left thoracic chain and
	Mar. 20	2.22	25.65	33.60	30.99	left splanchnic
3	Feb. 28	2.50	30.25			*Dec.* 20, 1938: left thoracic chain and left splanchnic
	May 16	2.84	31.88	40.80	27.98	*April* 12: abdominal chains + right splanchnic and semilunar ganglia
4	Mar. 3	1.88	32.50			*Feb.* 15: left thoracic chain, left splanchnic
	Mar. 6	2.00	31.03			
	Mar. 21	2.10		42.64	34.25	*Mar.* 22: abdominal chains, right
	Apr. 10	1.99	31.87	42.73	34.07	splanchnic
	Apr. 12	2.00		39.78	26.62	*May* 26: right thoracic chain
	June 9	2.50	30.41	38.28	25.88	
5	Feb. 27	3.07	30.84			*Jan.* 24: left thoracic chain and
	Mar. 1	2.99	30.18			left splanchic
	Mar. 3	3.10	28.25			*Mar.* 23: abdominal chains and
	Mar. 22	2.99	29.76	35.70	19.96	right splanchnic
	Apr. 11	3.02	27.72	34.73	25.28	*Apr.* 18: right thoracic chain
	May 31	3.10	28.15	37.12	31.86	
6	Feb. 27	2.24	35.14			*Feb.* 6: left thoracic chain and left
	Mar. 1	2.47	34.66			splanchnic
	Mar. 3	2.50	34.44			*Mar.* 24: abdominal chains and
	Apr. 10	2.10	30.76	40.26	30.88	right splanchnic
	June 7	2.82	31.58	41.73	32.14	*May* 19: right thoracic chain
7	Mar. 8	2.34	32.16			Cat pregnant
	Mar. 14	2.94	31.08	41.73	34.26	*Mar.* 24: a, abdominal chains and
	Mar. 16	3.09	31.62	41.88	32.44	both splanchnics + semilunar
	May 17	2.77	23.42	35.40	51.11	ganglia; b, hysterectomy
						May 23: right thoracic chain
	July 5	2.5	25.34	33.09	30.58	*June* 19: left thoracic chain

TABLE 1—*Concluded*

CAT NUMBER	DATE	WEIGHT	CALORIES PER SQ. M.		PERCENTAGE INCREASE B.M.R.	OPERATIONS (REMOVALS) 1939
			Basal	After peptone		
	1939	*kgm.*				
8	Apr. 22	3.71	29.43	41.28	40.26	*Nov.* 12, 1938: last stage of complete sympathectomy
	May 24	3.60	30.32	42.09	38.81	
9	Apr. 21	2.73	31.42	40.39	28.55	*Nov.* 23, 1938: last stage of complete sympathectomy
10	June 24	2.47	33.08	42.09	27.24	*June* 8: last stage of complete sympathectomy
	June 27	2.45	32.62	42.08	29.00	

of the animal was registered on the spirometer record; only perfectly quiet periods were taken as a basis for judgment. The Meeh formula was used to estimate body surface.

RESULTS. I. *Sympathectomized cats.* Of the 10 animals belonging to this group 7 had had complete, the other 3, only partial sympathectomy. Of the latter, 1 was deprived of the two abdominal chains, the splanchnics, the semilunar ganglia and the left thoracic chain (having only the right thoracic); from the other 2 the left thoracic chain and the corresponding splanchnic were removed. The b.m.r. was determined before any operation, and, as a rule, after each stage of sympathectomy, and, of course, after complete removal of the thoraco-lumbar chains. Always, the determinations were made only after the cats had entirely recovered from the operation.

In the completely sympathectomized animals the first study of the b.m.r. and the s.d.a. was usually undertaken 3 to 5 weeks after the final stage of the operation. In two instances, however (cats 8 and 9), the first measurements were made 5 months after completion of the sympathectomy.

The results obtained under the above conditions are summarized in table 1.

The weight of the animals during the experimental period (5 to 6 months) increased in nearly all cases.

The b.m.r. during the period was, with few exceptions, strikingly constant. There were a few slight decreases in the rate (i.e., from 5 to 10 per cent), decreases which, obviously, cannot be considered as abnormal.

After administration of Witte's peptone, the metabolic rate was regularly, without any exception, augmented between 19.96 per cent (cat 5) to 51.11 per cent (cat 7). Such increases have been noted in both the completely and partially sympathectomized animals.

II. *Action of tartrate of ergotamine (gynergen) on the s.d.a. in normal cats.* In these experiments, which serve as controls on the preceding group, 2 cats were used (table 2). The following results were obtained. A. In normal circumstances the metabolic rate, after peptone administration, was increased, as expected. B. The injection of gynergen (0.5 mgm. per kgm. body weight) does not induce any change of the rate. C. After simultaneous administration of gynergen and peptone a great increase

TABLE 2

Basal metabolic rates and maximal rates after administration of Witte's peptone and ergotamine (gynergen) in normal cats

CAT NUMBER	DATE	WEIGHT	CALORIES PER SQ.M.		PERCENTAGE INCREASE B.M.R.
			Basal	After peptone and gynergen	
	1939	*kgm.*			
11	Apr. 17	1.79	28.36	28.21*	
	Apr. 20	1.87	26.03	42.21	62.75
12	May 23	2.30	25.24	35.50	40.65
	June 5	2.69	26.44	44.76	69.29
	June 9	2.65	26.37	40.72	54.41

* Gynergen alone administered.

TABLE 3

Basal metabolic rates and maximal rates after administration of dial, Witte's peptone and gynergen

CAT NUMBER	DATE	WEIGHT	CALORIES PER SQ.M.		PERCENTAGE INCREASE B.M.R.
			Basal	After peptone and gynergen	
	1939	*kgm.*			
11	June 12*	2.42	27.20	40.98	50.66
	June 12†		27.20	36.09	32.66
12	June 12	2.72	26.50	44.31	69.00

* Readings 2 hours after the administration of peptone.

† The same, 4 hours later.

of the rate (40.65 to 69.29 per cent) takes place. D. Finally, the same experiment (C), performed under dial anesthesia (0.75 cc. per kgm.) has shown that the s.d.a. of the peptone is delayed but by no means suppressed (table 3).

COMMENTS. The discrepancies between the results here reported and those reported by investigators mentioned in the introductory paragraph may be due to differences in experimental conditions. For example, the

choice of animal is not without importance for study of s.d.a. of protein. It seems, indeed, that the rabbit is not a desirable animal for such experiments (cf. Schaeffer, Le Breton and Dontcheff), because it is naturally herbivorous and not accustomed to taking an abundance of protein, and also because its stomach always contains accumulated food.

Caution must be exercised in interpreting the action of ergotamine (gynergen). It is well known that the effective doses of the drug vary both with the animal and with the desired blocking of this or that function of the sympathetic system. On the other hand, the effects of ergotamine may not be limited to the sympathetic nervous system (Moore and Cannon, 1930; Ring, Dworkin and Bacq, 1931). The variability of the results obtained by different investigators may be thus explained. Noyons (1932) relates that "gynergen suppresses or *delays* in some cases the reaction." Schaeffer and his collaborators (Schaeffer and Le Breton, 1938) and also Rothschild (1938) have reported that the s.d.a. of protein is always suppressed, after administration of gynergen, the former in the rabbit and the latter in cats. In experiments here reported the same gynergen (0.5 to 0.7 mgm. per kgm. body weight) has never resulted in diminution or suppression of s.d.a. The results obtained from use of ergotamine, therefore, do not seem to be convincing, because of their variability. For that reason another method of excluding the sympathetic system was desirable. Noyons, as well as Schaeffer and Le Breton have used section of the splanchnics for that purpose. But simple section of two nerves, or even more, obviously, does not solve the problem. It is evident that complete removal of the thoraco-lumbar chains and ganglia is the best way of ruling out the sympathetic from any participation in s.d.a. Under these experimental conditions the phenomenon of the s.d.a. of proteins occurs quite as well as before. This fact itself ends the necessity for further discussion of various hypotheses of the relation of s.d.a. to the sympathetic nervous system.

The results detailed in the foregoing pages definitely indicate that the sympathetic system does not play any part in the producing of the s.d.a. of proteins.

SUMMARY

Cats were used, both sympathectomized and normal. In the former the b.m.r., during the experimental period, was constant. After administration of Witte's peptone, the metabolic rate was regularly, without any exception, augmented between 20 and 50 per cent. Such increases have been noted in both completely and partially sympathectomized animals (table 1).

In the normal cats, the injection of tartrate of ergotamine (gynergen)—0.5 to 0.7 mgm. per kgm.—does not suppress the s.d.a. of protein (table 2).

These results indicate that the sympathetic system does not play any part in the producing of the s.d.a. of proteins.

It is a pleasure to express my thanks to Prof. W. B. Cannon for suggesting the problem and for his interest and helpful advice during the course of these investigations.

I also wish to thank Miss S. Bevin, whose technical help has been invaluable to me.

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A QUALITATIVE STUDY OF NORMAL GONADOTROPIN[1]

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Intensive studies have been carried out on the gonadotropic substances present in the anterior pituitary, pregnancy and menopause urine and pregnant mare serum. Comparatively little work has been done on the gonadotropic substance excreted by normal women during the menstrual cycle. This substance, associated as it almost certainly is with ovulation, deserves study and no doubt the reason it has been neglected has been the difficulty of obtaining adequate amounts. In any given subject, one does not know exactly when excretion will occur, and the amount is so small that, after having demonstrated its presence, very little is left.

For the past three years the author has been engaged in assaying the urines of normal women and, during that period, day by day tests of some 70 complete menstrual cycles have been made. Many times, a small amount of precipitate in which activity had been demonstrated was left over and this pooled precipitate represents the material used in this study.

If this hormone is responsible for ovulation some knowledge of its properties is desirable. In the first place, if it should prove to be identical with one whose origin and properties were established, this knowledge might serve better to define its significance in respect to human ovulation. In the second place, sterility would be the expected result of its absence and the most logical treatment of such cases (since the amount which can ever be obtained from original sources is so small) would be dependent upon identifying it with some other, more readily available hormone. The purpose of this study, therefore, was to compare the gonadotropin occurring in the urine of normal women during the menstrual cycle with the four previously mentioned (anterior lobe, pregnancy urine, menopause urine and pregnant mare serum), to see which of these it most nearly resembled. Comparison was made on the basis of effects on ovarian weight, degree of luteinization, seminal vesicle weight and testicular development. Lack of material renders the investigation incomplete, especially quantitatively; such desirable studies as attempts at purifica-

[1] This investigation was supported by a grant from the Penrose Fund of the American Philosophical Society.

tion, fractionation, the use of hypophysectomized animals, etc., could not be carried out because of this limitation.

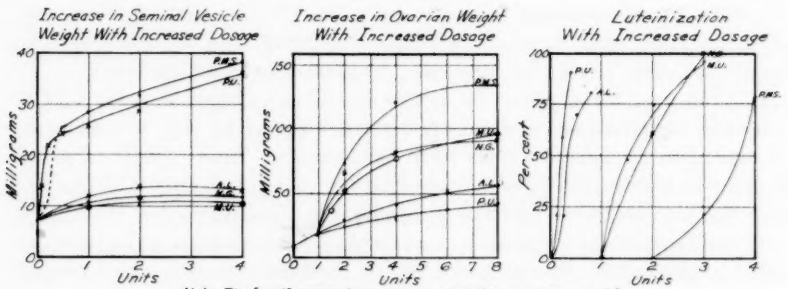
EXPERIMENTAL. The five preparations studies were as follows:

1. *Normal gonadotropin* (N.G.). A pool of left-over precipitates from 50 positive reactions occurring at the time of presumed ovulation, from 15 subjects. These precipitates were prepared by the tannic acid method of Levin and Tyndale (1).

2. *Menopause urine* (M.U.). A pool of the precipitates of 24-hour urines, from 19 women between the ages of 54 and 80, also prepared by the tannic acid method.

3. *Pregnant mare serum* (P.M.S.). The preparation used was "Gonadin," kindly supplied by Dr. Wonder, of the Cutter Laboratories.

4. *Pregnancy urine* (P.U.). A pool of 108 individual samples from all stages of pregnancy, prepared by the alcohol precipitation method of Wallen-Lawrence and Van Dyke (2).



Note. The 'unit' = amount necessary to double ovarian weight

-FIGURE I-

5. *Anterior pituitary* (A.L.). Acetone-dried sheep pituitaries extracted by the sodium acetate-acetic acid method of Wallen-Lawrence and Van Dyke (2).

The unit chosen as a basis for comparison was the amount of each necessary to double the ovarian weight of 21-day-old rats, injecting once per day for 3 days and killing at the end of 100 hours. The amount of each (total dose) equivalent to one "unit" was: N.G. 26 mgm., M.U. 7.5 mgm., P.M.S. 10 labelled units, P.U. 8 mgm., A.L. 2 mgm.

Having determined the "unit" for each preparation, multiples of the unit were injected to obtain the information presented in figure 1. Each point on the graphs represents the average of 10 rats, except for N.G., for which only 5 were used.

RESULTS. 1. *Seminal vesicle weights.* These results are shown in the first graph. It will be noted that the effect of increasing the number of "units" injected is approximately the same for A.L., M.U. and N.G.

2. *Ovarian weights.* The middle graph presents the results of increased dosage on ovarian weights. It will be noted that the M.U. and N.G. curves closely parallel each other.

3. *Degree of luteinization.* The increased luteinizing effect of increased dosages is shown in the third graph. Again it will be seen that the effects of N.G. and M.U. are practically identical.

4. *Testicular development.* Beginning on the 21st day of life groups of males were injected daily with one-third unit of each preparation. Pairs were killed at intervals of 5 days up to the 46th day. Testes and seminal vesicles were weighed and the testes sectioned. The results, as compared to similar groups of controls, were as follows: P.M.S. and P.U. accelerated the growth of seminal vesicles throughout, P.M.S. to the greatest degree, A.L., M.U. and N.G. did not. Testicular weight was somewhat increased in all, to the greatest degree by A.L. P.M.S. and P.U. stimulated the development of interstitial tissue, P.M.S. to the greatest degree, the others did not. Spermatogenesis was not accelerated as regards time, the sections of testes of all groups showed at least some sperm heads after 15 days' injection (36th day of life), and all showed sperm after 25 days' injection (46th day of life). The rate of early stages of spermatogenesis appeared to be increased by A.L., M.U. and N.G., as indicated by larger tubules and by the numbers of mitotic figures visible; this was not true of P.M.S. and P.U.

Discussion. It will be noted that by all tests performed, the resemblance between normal gonadotropin and menopause urine gonadotropin is striking, and the tentative conclusion that they may be identical appears justified. The organ of origin of menopause urine gonadotropin is believed to be the anterior lobe of the hypophysis (3). (The "beliefs" referred to in this discussion are those expressed in recent reviews. The author recognizes that complete unanimity of opinion does not exist.) In the present study, M.U. and N.G. are seen to resemble the crude anterior lobe preparation very closely as regards their effects on males, the luteinizing effect of A.L. on females is greater. If it is accepted that N.G. is identical with M.U. and also originates in the anterior lobe, the results of a number of other studies can be brought to bear upon the problem of human ovulation. These results are:

1. The author has made day by day assays, on the same urines, for both estrin and gonadotropic hormone content, of 18 complete menstrual cycles. These results will soon be published, but a most significant finding may be mentioned here. Invariably, the rise in estrin excretion which occurs toward the mid-interval of the cycle, *precedes* the sharp peak of gonadotropin output. Three typical cycles are shown in figure 2. The second rise in estrin secretion, usually not followed by a positive gonadotropic response, requires further study. Tentatively it may be suggested that

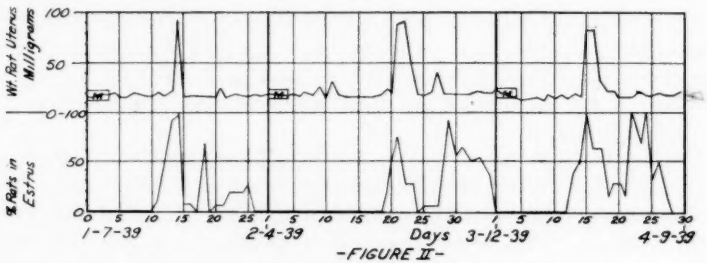
its failure to stimulate hypophysial activity is due to the inhibiting effect of progestin.

2. Estrin has been shown (4) to stimulate luteinizing hormone secretion by the pituitary.

3. The presence of both follicle-stimulating and luteinizing hormone is believed to be necessary for ovulation (5, 6).

4. Small amounts of luteinizing hormone greatly increase the effect of follicle-stimulating hormone on the ovaries of the immature rat (7, 8).

Putting these pieces together, one obtains the following as a working hypothesis (no more) of the mechanism of ovulation. The anterior lobe secretes follicle-stimulating hormone which maintains the normal growth of the follicles. As a follicle ripens it produces estrin. The estrin stimulates L.H. production on the part of the pituitary and the combination of L.H. and F.S.H. produces ovulation. This combination, being more effective on the test animal's ovaries than F.S.H. alone, gives the marked response which characterizes a positive reaction.



-FIGURE II-

With the discovery that P.M.S. is apparently a complete, or nearly complete, anterior lobe replacement substance, and is capable of bringing about ovulation in a number of species of animals (9), the development of clinically useful preparations of menopause urine gonadotropin becomes of less consequence. P.M.S. is, however, certainly not identical with A.L. and its marked action on interstitial tissues and consequently upon accessory sex structures might be a disadvantage when used in the male.

As regards the comparisons of the better known hormones, in view of the fact that quantitative studies on the intact animal are notoriously misleading, little need be said. If, as is commonly believed (10), L.H. is also the interstitial cell stimulating hormone, it is apparent that L.H. from chorionic sources is much more active than L.H. from anterior lobe. On the other hand, the greater effect of P.M.S. on seminal vesicles, with apparently much less L.H. present than P.U. contains, might argue in favor of the existence of another, specific, I.C.S. hormone, as is believed to be the case by Evans (11). The failure to hasten spermatogenesis

by the use of any of these hormones is in agreement with the work of Moore (12).

SUMMARY

A qualitative comparison of the effects of the gonadotropic substance present in the urine of normal women at the presumed time of ovulation, with gonadotropins from menopause urine, pregnancy urine, anterior lobe and pregnant mare serum have been made. The bases of comparison were effects on ovarian weight, luteinization, seminal vesicle weights and testicular development. By all tests, normal gonadotropin was found to be strikingly similar in its effects to menopause urine. On the basis that the latter originates in the anterior lobe, plus certain other findings, a tentative explanation of the mechanism of ovulation in the human is suggested.

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THE ORIGIN OF RESPIRATORY RHYTHMICITY[1]

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Concepts of the origin of respiratory rhythmicity fall into two categories: the one, that the respiratory center is inherently rhythmical; the other, that rhythm is imposed on the center by some mechanism external to it. Most modern texts of physiology and recent reviews of the neural control of respiration (Finley, 1931; Cordier and Heymans, 1935; Heinbecker, 1937) are inclined toward the former view, though evidence for the latter is by no means slight.

Those favoring autochthonous rhythmicity of the respiratory center have been led to this conclusion by the persistence of rhythmic respiration after more or less complete deafferentation of the center. Outstanding among these investigations are the experiments of Rosenthal (1862), Winterstein (1911), Heymans and Heymans (1927), and Adrian and Buytendijk (1931). Rosenthal removed the fore part of the brain and sectioned the cranial nerves of the rabbit; Winterstein studied action potentials in the central stump of the sectioned phrenics of the curarized dog on cessation of artificial respiration; Heymans and Heymans studied laryngeal movements of the isolated perfused head of the dog; and Adrian and Buytendijk studied potentials originating in the vagal lobes of the extirpated brain stem of the goldfish. All noted the persistence of rhythmic respiratory activity and assigned it to inherent rhythmicity of an isolated respiratory center.

The opposing view, that rhythm is imposed on the respiratory center by mechanisms external to it, is based on more positive evidence, namely, the loss of rhythmicity on exclusion of these external sources. Coombs and Pike (1918) and Sharpey-Schafer (1932) maintained that vagal lung stretch and dorsal root proprioceptive stimuli are of prime importance in rhythmic reflex drive of the respiratory center. More convincing, however, is the work of Marckwald (1887, 1890) which demonstrated in the brain stem above the medulla, a center which rhythmically inhibits the medullary respiratory center. The vagus exerts a similar reflex effect. With the exclusion of this center and the rhythmic reflex drive of the vagus there

[1] Aided by a grant from the Rockefeller Foundation.

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develops a cramp-like inspiration, tonically maintained with cessation of rhythmic respiration. Marckwald placed this center in the inferior colliculus. Lumsden (1923) redescribed this region as the "pneumotaxic" center but failed to note the important rôle of the vagus. Lumsden placed this center in the upper pons. Henderson and Sweet (1929) and Hess (1931) maintained that inspiratory cramps or apneuses were manifestations of decerebrate rigidity due to exclusion of the red nuclei, and were not respiratory, but postural chest disturbances. Stella (1938a, b) and Pitts, Magoun and Ranson (1939b) have demonstrated clearly that the inspiratory cramps of Marckwald or apneuses of Lumsden are respiratory responses of a medullary inspiratory center freed from rhythmic drive of two systems, one a vagal reflex system, the other the brain stem pneumotaxic center. Stella (1938a) has placed the penumotaxic center in the upper pons as did Lumsden.

In view of the importance of this brain stem mechanism in imparting rhythmicity to respiration further study seemed desirable as to its site, pathways of connection with the respiratory center, and significance in the respiratory rate changes of panting and pain polypnea. These problems are considered in the present study.

Methods. Our experiments have been carried out on cats anesthetized with 20 mgm. of nembutal per kilo given intravenously; 1 gram of urethane per kilo given intraperitoneally; or decerebrated under ether anesthesia, and allowed to recover for an hour or more before use. Respiration has been recorded with a closed circuit spirometer intermittently refilled with oxygen, the carbon dioxide being absorbed by a soda lime tube connected to the tracheal cannula. Lesions have been made in two ways: one, by transection or partial section with a flattened bent wire after suitable exposure of the fourth ventricle by removal of the cerebellar vermis; the other, by placing of multiple electrolytic lesions with electrodes oriented in the Horsley-Clarke stereotaxic instrument. For the most part bipolar needle electrodes were used with points separated 2 mm. A current of 3 ma. for 60 seconds repeated every 2 mm. vertically, or every 1 mm. laterally, led to cavitation with a thin surrounding rim of tissue which did not stain with myelin sheath stains. All lesions have been identified in serial sections of the brains stained by the Weil method. Alternate sections were stained with cresyl violet in those experiments in which identification of the red nucleus was important.

Electrolytic lesions have the advantage of discreteness, proper localization, and the ability to increase in size regularly and progressively. The disadvantage lies in the necessity for frequent changing of electrodes due to electrolysis, and to probable extension of the zone of damage beyond the lesion visible in stained sections. This latter disadvantage is probably equally true of blunt knife sections. For this reason we shall lay special

emphasis on negative results; i.e., if a given lesion does not affect respiration, then the region identified in stained sections as destroyed by the lesion is not *fundamentally* important. Where positive results are presented as significant, they have been repeated many times. The vagal reflex influence on respiration has been temporarily abolished by cold blocking of the vagi with thermodes described in our previous communication. In the figures presented, the outline drawings of the brain stem were made by tracing the projected image of the section. The spirometer records have been trimmed and the slope removed in the interest of space conservation; in all records the downstroke indicates inspiration. Time in all records indicates 6 second intervals.

The general plan of experimentation has been to record respiration and the effects of bilateral cold blocking of the vagi in the intact animal, to note the respiratory drive of the brain stem mechanism alone. Lesions were made and the cold blocking of the vagi repeated, to note what effects the lesion may have had on the brain stem pneumotaxic mechanism. It must be emphasized again that only with exclusion of the vagal influence by cold blocking (or section) can alteration in the brain stem mechanism be brought out. In all the records, the period of vagal block is indicated by the white line beneath the respiratory record.

RESULTS. *The site of the brain stem pneumotaxic center.* It is common knowledge that animals which have been subjected to an intercollicular decerebration maintain intact a central respiratory mechanism which responds in a normal way to carbon dioxide increase, oxygen lack, sensory nerve stimulation, and vagal section. On the other hand animals which have been decerebrated at a pontine level, though they maintain a normal type of respiration and respond normally to chemical and peripheral nerve stimulation, show a striking response on bilateral section of the vagi (Marckwald, 1887; Stella, 1938a, b; Pitts, Magoun and Ranson, 1939b). This response consists of a tonic inspiration maintained for seconds to minutes with complete cessation of rhythmic respiration. It may be interrupted occasionally by brief expirations, but unless some form of artificial respiration is maintained the animal soon deteriorates and dies. If on the other hand the vagi are cold blocked for short periods (Stella; Pitts, Magoun and Ranson) the animal may be maintained in good condition for a considerable period of time.

The levels of transection which, combined with vagal block, have resulted in inspiratory cramp or apneusis are shown in figure 1 on dorsal and ventral views of the brain stem of the cat with the cerebellum removed. The upper end of the drawings indicates the approximate level of the usual intercollicular decerebration. The heavy transverse lines labeled 1 to 6 indicate the dorsal and ventral limits of pontine sections which resulted in apneuses in 6 cats when the vagi were cold blocked. Examples of apneuses

are shown in figures 2A3, 2D3, 3A2, 3B3, etc. It follows then that between the cephalic end of the sketches shown and the levels of transection, there is present a brain stem mechanism which is capable of maintaining rhythmic respiration when the sensory inflow from the vagus is blocked. This

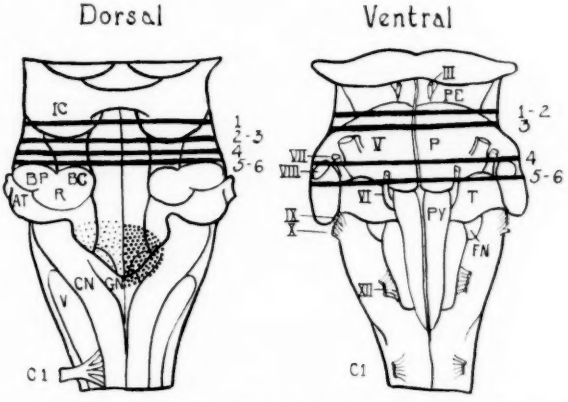


Fig. 1. Dorsal and ventral views of the brain stem of the cat with the cerebellum removed to show the levels of section which, combined with bilateral section or cold blocking of the vagi, lead to apneusis. The cephalic extent of the drawings indicates the level of the usual intercollicular decerebration, which combined with vagal block does not lead to apneusis. Levels 1 to 6 indicate transections in 6 cats which did lead to apneusis on vagal block. The brain stem pneumotaxic center lies between section 1 and the cephalic limit of the figures. The inspiratory division (right) and the expiratory division (left) of the respiratory center are indicated by the stippled areas projected on the dorsal view of the floor of the fourth ventricle. They are each indicated unilaterally to avoid the confusion of overlapping.

A, aqueduct; *AT*, acoustic tubercle; *BC*, brachium conjunctivum; *BCX*, decussation of brachium conjunctivum; *BIC*, brachium of inferior colliculus; *BP*, brachium pontis; C_1, first cervical nerve; *CG*, central gray; *CN*, cuneate nucleus; *DT*, descending trigeminal root; *FN*, facial nucleus; *GF*, genu of facial nerve; *GN*, gracile nucleus; *IC*, inferior colliculus; *IP*, interpeduncular nucleus; *LL*, lateral lemniscus; *MET*, mesencephalic trigeminal root; *ML*, medial lemniscus; *MLF*, medial longitudinal fasciculus; *MT*, motor trigeminal nucleus; *NDT*, nucleus of descending trigeminal root; *ON*, oculomotor nucleus; *P*, pons; *PE*, cerebral peduncle; *PY*, pyramid; *R*, restiform body; *RN*, red nucleus; *S*, tractus solitarius; *SC*, superior colliculus; *SN*, substantia nigra; *SO*, superior olive; *ST*, sensory trigeminal nucleus; *T*, trapezoid body; *V*, tuberculum cinereum; *III* to *XII*, corresponding cranial nerves.

region is separated from the medullary inspiratory and expiratory centers by a distance of at least 10 millimeters. These centers which have been localized by Pitts, Magoun and Ranson (1939a) using the Horsley-Clarke technique are shown projected on the dorsal view of the fourth ventricle as stippled areas. It is evident that these centers in the absence of an

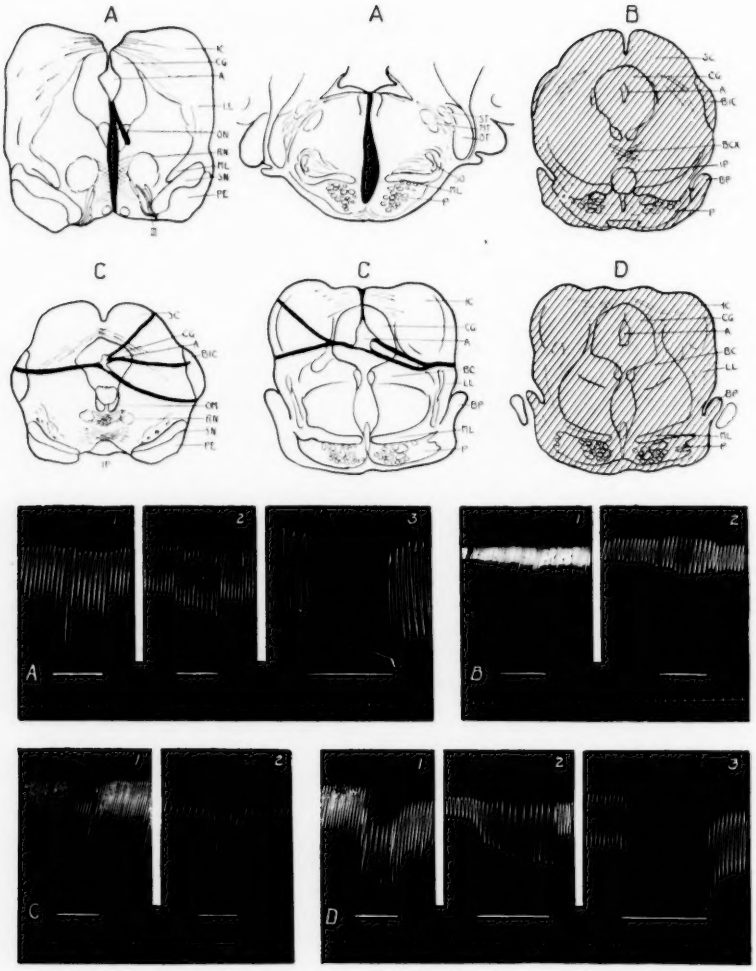


Fig. 2. Brain stem lesions in the cat which do not produce apneusis on cold blocking of the vagi. In the respiratory records below, inspiration is indicated by the downstroke of the spirometer record; the white line indicates the period of application of the cold block to the vagi; the time record shows 6 second intervals; record 1 indicates the response prior to the lesion; record 2 indicates the response after the lesion shown in the upper part of the figure; and record 3 indicates the apneusis produced by subsequent lesions in the pons similar to those shown in figure 3. In cat A, a midsagittal section destroying decussating fibers from the level of the anterior commissure to the middle of the pons did not produce apneusis. In cat C, removal of the mesencephalic tectum did not produce apneusis. In cats B and D, transections more than 1 and 2 mm. respectively caudal to the red nucleus did not produce apneusis. The apneuses shown in figure A3 and D3 resulted from vagal block after subsequent pontile lesions, not figured, but similar to those of figure 3.

intact vagal mechanism, cannot alone maintain rhythmic respiration, though in conjunction with either the vagus or the upper brain stem mechanism, respiration is rhythmic and relatively normal.

Between the limits indicated in figure 1 for the site of the brain stem pneumotaxic mechanism lie 3 structures which have been assigned this rôle: the inferior colliculi, the red nuclei and the tegmentum of the cephalic part of the pons.

The red nucleus. Henderson and Sweet (1929) maintained that apneusis resulted from exclusion of the tonus inhibiting mechanisms of the red nuclei and vagi; that in fact, apneusis is but a decerebrate postural response of the chest unrelated to respiration. In 4 animals midsagittal sections through the mesencephalic region were made in such a position as to interrupt decussating fibers of the red nuclei. The 2 sketches shown in figure 2A indicate the completeness of one of these sagittal sections, which extended from the anterior commissure to below the middle of the pons. The respiratory records in figure 2A1 before, and 2A2 after the midline lesion indicate clearly that the brain stem pneumotaxic center is functional in both instances, as evidenced by rhythmic respiration during cold block of the vagi (the duration of the block being indicated by the white line beneath the record). The apneusis in 2A3 was produced by vagal block following the placement of bilateral pontine lesions similar to those shown in figure 3 (to be discussed below). In animals represented by B and D of figure 2, a transection of the brain stem was made in which the plane of section was caudal to the red nucleus; in B, more than 1 mm.; and in D, more than 2 mm. caudal to the red nucleus. Neither animal was apneustic on vagal block, as shown by figures 2B1 and 2D1 before, and 2B2 and 2D2 after the transection. The apneusis in figure 2D3 resulted in vagal block after pontine lesions as shown in figure 3. Two other animals gave similar results. It is evident that in these 8 animals, exclusion either of the decussating fibers of the rubrospinal tracts (4 animals) or of the red nuclei themselves (4 animals) did not inactivate the brain stem pneumotaxic mechanism. The pneumotaxic region thus lies caudal to the red nuclei and no essential fraction of its emergent fibers decussate.

The inferior colliculi. Marckwald (1887) maintained that the brain stem mechanism inhibiting the inspiratory cramp after section of the vagi lay in the inferior colliculi. In 3 animals in which the mesencephalic tectum was undercut by a posterior approach after removal of the cerebellar vermis (as shown in fig. 2C), there was no evidence of apneusis on vagal block; see figure 2C1 before, and 2C2 after the tectal undercutting. In one animal apneusis was produced on vagal block after midsagittal section and undercutting of the colliculi. Another animal survived the combined section without apneusis. It is thus evident that removal of the colliculi does not inactivate the brain stem pneumotaxic mechanism.

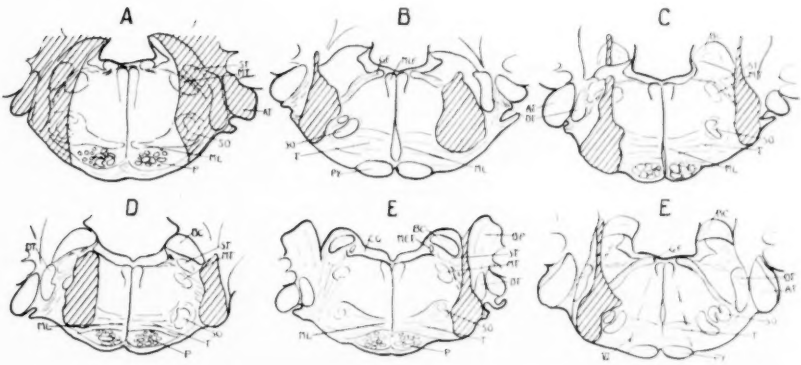
The tegmentum of the pons. Lumsden (1923) and Stella (1938a) both place the pneumotaxic center within the upper 2 or 3 mm. of the pons. A comparison of figures 1 and 2 leads us to essentially the same conclusion. Since apneusis is not produced on vagal block with a level of transection shown in figure 2D no essential part of the mechanism lies cephalic to this level.[3] The plane of section in figure 2D, made in front of the tentorium, is quite different from the planes of section shown in figure 1, which were made behind the tentorium. The ventral extent of the sections in figure 1 (sections 1, 2 or 3) is not essentially different from that in figure 2D. But dorsally the section in figure 2D begins at the junction of the superior and inferior colliculi and is thus several millimeters cephalic to section 1, figure 1. Since the mesencephalic tectum has been ruled out as significant, figure 2C; and since the basilar part of the pons is similarly not essential, figure 2D; the essential part of the pneumotaxic center must be localized to the tegmentum of the upper few millimeters of the pons.[4] Attempts to localize the pneumotaxic center more closely than this have to date been unsuccessful owing to inability to remove the tentorium and maintain the animal in good condition.

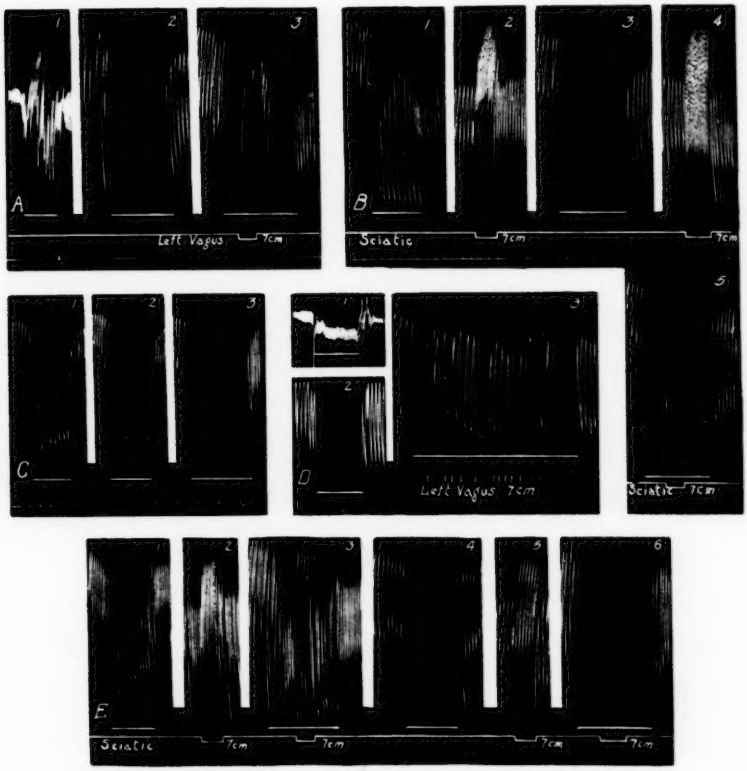
Pathways connecting the pneumotaxic and respiratory centers. Pitts, Magoun and Ranson (1939b) have presented evidence showing that the

[3] It is possible that the pneumotaxic mechanism is spread out over a considerable extent of the tegmentum of the mesencephalon and upper pons, but that its caudalmost part is sufficient to impart rhythmicity to respiration after elimination of its more cephalic portions by transection.

[4] It is possible that the transections shown in figure 1, sections 1, 2 and 3 inactivate neural tissue a few millimeters caudal to the section. Thus the transections might not actually be caudal to the entire pneumotaxic region, but rather inactivate it by passing through it, or just cephalic to it. Sections 4, 5 and 6 must certainly be caudal to it.

Fig. 3. Lesions of the lower brain stem of the cat which interrupt the descending pathways from the pneumotaxic center to the respiratory center and thus lead to apneusis on cold blocking the vagi. In A, the lesions were made with a blunt knife; in B to E, the lesions were electrolytic ones made with the Horsley-Clarke instrument. In the respiratory records below, the white line indicates the period of vagal block; the signal record indicates stimulation of vagus or sciatic with a Harvard inductorium with secondary coil at 7 cm. In respiratory records A and D, the lesions shown above were placed between A1 and A2, and between D1 and D2; in respiratory record C, the right lesion was placed between C1 and C2, and the left lesion was placed between C2 and C3; in B, the lesions were placed between B2 and B3; and in E, between E3 and E4. In each animal these bilateral lesions destroying the ventrolateral tegmentum produced apneusis on cold blocking of the vagi. Stimulation of the central end of one cut vagus during the apneusis produced by block of the other, A3 and D3, leads to prompt expiration. Stimulation of the sciatic, which leads to polypnea during rhythmic respiration, B2, B4, E2, E3, E5, leads only to a slight inspiratory decrease, E6, or inspiratory increase, B5, during apneusis. Decorticate panting, A1 and D1, is abolished by the pontile lesions, A2 and D2.


A
B
C
D
E
E


A
Left Vagus
7cm
B
Sciatic
7cm
7cm
C
D
Left Vagus 7cm
Sciatic 7cm
E
Sciatic
7cm
7cm
7cm
7cm

inspiratory apnea of typical apneusis is a response of the medullary inspiratory center, freed from the inhibiting influences of the vagi and the pneumotaxic center. In a preceding paper (1939a) they localized the inspiratory and expiratory divisions of the respiratory center to the ventral and dorsal reticular formations underlying the lower part of the fourth ventricle. Since a distance of more than 10 mm. separates the pneumotaxic center and the medullary inspiratory and expiratory centers, the location of the pathways connecting them becomes a matter of interest. Preliminary experiments indicated that the pathways were laterally situated. A series of lesions were placed destroying portions of the pons and upper medulla to delimit the pathways more definitely.

In figure 3, A to E, are shown sections of the pons and medulla from 5 cats in which the lesions are indicated by the diagonal hatching. The lesions of cats B to E were electrolytic ones, placed with the aid of the Horsley-Clarke instrument. In E, the lesions were asymmetrical cephalocaudally and required two figures to indicate their location. In A, the lesions were made by knife section after removal of the cerebellar vermis. The effect of vagal block before the lesions is shown in figure 3, A1, B1, C1, D1 and E1. After the lesions cold block of the vagi produced apneusis in all instances as shown in figure 3, A2, B3, C3, D2 and E4. A lesion on the right side only of the brain stem (fig. 3C, right side) combined with bilateral vagal block did not produce apneusis but only a greater degree of slowing and inspiratory prolongation (fig. 3C2) than before. After the added left sided lesion of 3C, typical apneusis (fig. 3C3) occurred on vagal block. Thus a unilateral lesion combined with bilateral vagal block does not produce apneusis, the remaining functional half of the pneumotaxic mechanism being sufficient to impart rhythmicity to respiration. This fact has been observed in a number of instances. Similarly bilateral destruction of the pathways with unilateral vagal block does not lead to apneusis.

To rule out the possibility that apneusis results from a nonspecific effect of trauma, centrally placed lesions were made. In figure 4A, the 3 sections from one animal indicate the extent to which the central portion of the tegmentum may be destroyed without seriously affecting respiration. In this animal multiple large electrolytic lesions produced cavitation extending from the caudal limits of the inferior colliculi to the middle of the pons. In figures 4A1 and 4A2 are shown the results on cold blocking of the vagi before and after the lesions. It is evident that the pneumotaxic center or its projections have suffered no essential damage. Figures 4, B, C and D represent medial tegmental destruction in 3 animals which had been decerebrated at a high level under ether and exhibited a marked degree of decorticate panting. The corresponding kymograph records B1, C1 and D1 indicate response to vagal block before; and B2, C2 and D2,

after the lesions. In each case panting continues throughout and there is no suggestion of apneusis. Thus no essential part of the pathways passes through the central portion of the brain stem.

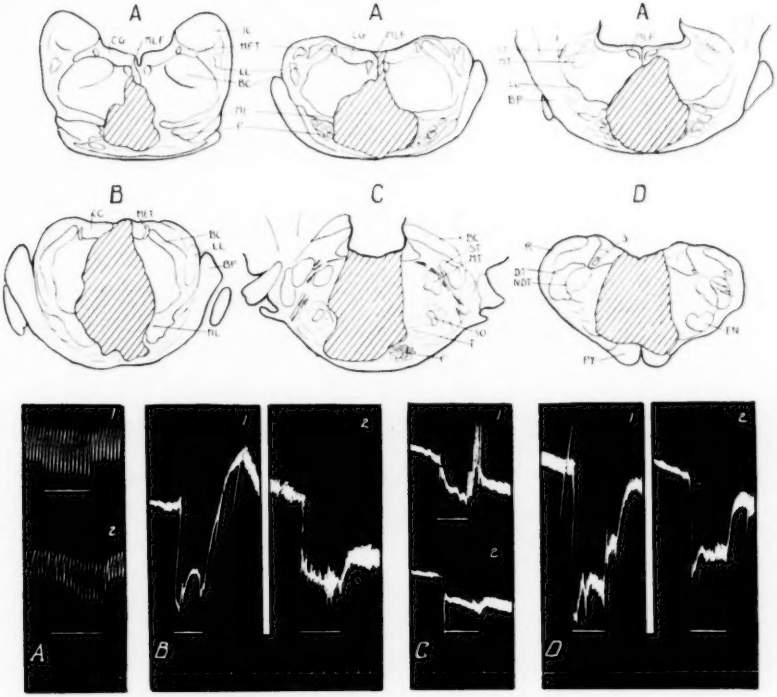


Fig. 4. Lesions of the central part of the tegmentum of pons and medulla which do not abolish decorticate polypneic panting, and which do not lead to apneusis on bilateral vagal block. In cat A, the lesions were continuous electrolytic ones producing cavitation extending from the caudal end of the inferior colliculi to the middle of the pons; in cats B, C and D, the lesions were made by blunt knife section. Respiratory records A1, B1, C1 and D1 were made before the lesions; and A2, B2, C2 and D2 were made after the lesions. The period of vagal cold block is indicated by the white line. Cats B, C and D showed marked decorticate polypneic panting which persisted unchanged after the sections. Note that while there is a marked shift of the respiratory midposition toward the inspiratory side in records B to D during vagal block, panting continues without appreciable alteration.

Figures 3 and 4 demonstrate clearly that the pathways connecting the pneumotaxic with the respiratory centers course laterally through the pons and upper medulla. Furthermore closer inspection of figure 3 indicates that the tracts lie quite ventrally (note fig. 3E, the smallest of the lesions producing apneusis). In figure 5 are shown sections from 3 animals in

which the lesion is evidently incomplete on one side. In figure 5A, lesions were made in the lateral pons with a blunt knife. The lesion on the right side is quite incomplete laterally. Figure 5A1 before, and 5A2 after the lesions indicate the incomplete elimination of the brain stem pneumotaxic mechanism since respiration in figure 5A2, though showing a tendency toward apneusis becomes regularly rhythmic during the last third of the

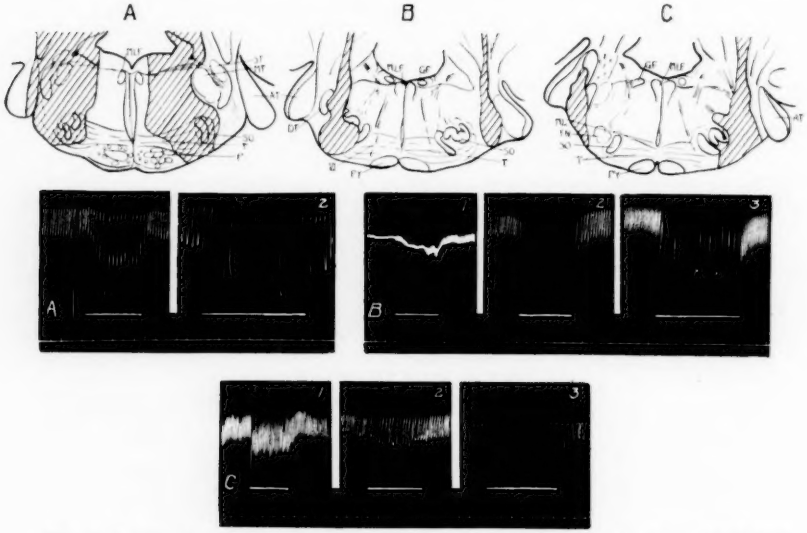


Fig. 5. Incomplete lesions of the ventrolateral tegmentum of the pons which lead to respiratory alteration without the production of complete permanent apneusis on vagal block. In A, the lesions were made with a blunt knife; in B and C, the lesions were electrolytic ones made with the Horsley-Clarke instrument. In the respiratory records, the lesions were placed between A1 and A2, and between B1 and B2. In C, the right lesion was placed between C1 and C2 and the left between C2 and C3. In A2 the apneusis produced by vagal block is incomplete with return of slow rhythmic respiration during the last third of the block. In B, polypneic panting was abolished and apneusis produced by the lesions, B2. Five minutes later slow panting movements returned and apneusis on vagal block was incomplete, B3. In C, polypneic panting was slowed by a right lesion, B2, and abolished by the left lesion, B3, but apneusis did not result at any time from vagal block.

period of vagal block. In figure 5B the lesion (electrolytic) on the right side is smaller and slightly more laterally placed than that on the left. Figure 5B2 indicates, however, that the brain stem mechanism was functionally eliminated temporarily, for apneusis resulted on vagal block. However, on retesting 5 minutes later apneusis was shown to be incomplete (fig. 5B3). In figure 5C, the lesion on the left side is small and far laterally placed. As shown in figures 5C1 before any lesion, 5C2 after the right

lesion and 5C3 after the left lesion, the pneumotaxic brain stem mechanism remained intact sufficiently to maintain rhythmicity during vagal block.

These results would indicate that the location of the descending pathway from the pneumotaxic center to the respiratory center is in the ventral part of the lateral tegmentum, ventral and slightly medial to the descending root of the trigeminal nerve, and that the pathway is quite discrete.

Pathways mediating decorticate panting. Thermal panting is difficult to obtain with any degree of regularity under nembutal anesthesia and impossible to obtain in our experience with intercollicular decerebration, though described by Keller (1930). However, decorticate polypneic panting may be regularly obtained by a high decerebration performed under ether. With the completion of a decerebration sparing the major part of the hypothalamus intense panting usually begins and continues unabated for an hour or more. What relation such decorticate panting has to thermal panting, if any, we do not know, but certain similarities exist. The polypnea, tongue, nose and jaw movements are the same. Furthermore, decorticate polypnea may be reinforced by placing the animal on a heating pad, though panting continues with even a subnormal rectal temperature. The following experiments have been performed on animals showing decorticate panting. In figure 2, cat B panted prior to, but not after, the intercollicular transection shown (note fig. 2B1 before, and 2B2 after the section). It is to be noted that panting continued during vagal block in 2B1. Thus it is probable that some higher lying centers exert an accelerating influence on the pneumotaxic center, which influence is cut off by intercollicular decerebration. In figure 3, cats A and D similarly panted prior to the placing of lesions (3A1 and 3D1) while after the bilateral pontile lesions which destroyed the descending pathways from the pneumotaxic center (3A2 and 3D2), the animals ceased to pant. In 3 other animals similar lesions stopped the panting and produced apneusis when the vagi were blocked. In figure 4, cats B, C and D panted both before and after lesions which destroyed the central part of the tegmentum of the pons and medulla. In these animals it is equally evident that panting movements as well as the polypnea persist during vagal block with the central part of the tegmentum destroyed. From these results it would appear that decorticate polypneic panting is mediated through the pneumotaxic center and its descending pathways, and that some higher lying mechanism (above the level of an intercollicular decerebration) by facilitating the activity of the pneumotaxic center, accelerates the respiratory rhythm.[5] Although the vagal reflex mechanism exerts some tonic

[5] The possibility exists that the descending pathways for polypneic panting and for pneumotaxis are merely coextensive, panting not being mediated through the pneumotaxic center but by direct paths. We have, however, not been able to separate the two mechanisms.

effect as evidenced by the marked shift toward an inspiratory midposition on vagal block (figs. 4B, C and D), such vagal reflexes are not essential for the polypnea of decorticate panting. A further interesting confirmation of this view is obtained from cat B of figure 5. This animal, panting during the control periods of the experiment (fig. 5B1), ceased to pant after the placing of the bilateral lesions (fig. 5B2). However, some few minutes later slow panting movements began again, and on cold blocking of the vagi, apneusis was no longer complete (fig. 5B3). Thus recovery of panting and of the activity of the pneumotaxic mechanism occurred simultaneously. One exception has, however, occurred. In figure 5, cat C, panting occurred during the control periods (fig. 5C1), was considerably slowed after the lesion was made on the right side (fig. 5C2), and completely suppressed after the left side lesion (fig. 5C3). This animal was not apneustic on cold blocking of the vagi. It is possible that panting was about to cease anyway (at the start panting was slow), or that there is a quantitative relation between the functional remnant of the pneumotaxic pathway and the rate of respiration (only a small part of the pathway active in 5C3 after bilateral lesions).

Mechanism involved in pain polypnea. The reflex polypnea obtained on central stimulation of a cut sensory nerve in a decerebrate or lightly anesthetized animal is common knowledge. In figure 3B2 and 3E2 are shown examples of the polypnea resulting from central stimulation of the sciatic with a Harvard inductorium, the secondary coil being set at 7 cm. (1.5 volts in the primary). The increased rate and increased amplitude, especially of expiration, is typical. Following the placement of lesions (fig. 3B and 3E) which interrupt the descending pneumotaxic pathways, sciatic stimulation again leads to typical polypnea (figs. 3B4 and 3E5). However, if during the apneusis produced in these animals by vagal block, the sciatic is again stimulated, no polypnea results, only an increase in the apneusis (fig. 3B5), or a slight decrease in the apneusis (fig. 3E6). In two other similar experiments, increased apneusis in one, and slightly decreased apneusis in the other, resulted from sciatic stimulation. It is thus evident that with the pneumotaxic center eliminated, reflex pain polypnea may occur if the vagal mechanism is intact, but if both vagi and center are bilaterally eliminated (i.e., during apneusis), no polypnea results (in fact no rhythmic respiration). Whether pain polypnea may be mediated through the pneumotaxic center alone is not definitely answered from our observations. In figure 3E3 the sciatic was stimulated during vagal block prior to placing the lesions (i.e., with the pneumotaxic center intact). The control rate was rapid and the increase in expiratory amplitude so great that little opportunity for a rate increase exists. Such has been the case in other experiments. However, the increase in expiratory amplitude is much the same as in the normal.

Contrasted with the results on sciatic stimulation is the prompt expiration produced on central vagal stimulation during apneusis (fig. 3A3). There is a considerable after-effect from stimulation and a brief period of respiratory rhythmicity, during which the apneusis builds to its former level. In figure 3D3, intermittent brief stimulation of the vagus leads to intermittent inhibition of the apneusis and a relatively rhythmic respiration, a fact emphasized in our previous communication.

Discussion. The question as to whether rhythmic respiration results from automatic processes inherent in the respiratory center or not, resolves itself into two parts. Is activity within the center spontaneous; and is that activity spontaneously rhythmical? From the work of Marckwald; Stella; Pitts, Magoun and Ranson and the experiments reported above, the latter question may be answered in the negative. Rhythmicity is imposed on the medullary respiratory center by two bilateral mechanisms: the one, a vagal pneumotaxic mechanism; the other, a brain stem pneumotaxic mechanism located in the upper few millimeters of the pons. If the connections of both these mechanisms with the medullary respiratory center are interrupted, respiratory rhythmicity is lost; and in place of rhythmic respiration, a tonic maintained inspiration ensues (apneusis). If there remains intact a connection of one vagus, or a part of one descending pneumotaxic pathway with the respiratory center, its action becomes rhythmical. These two mechanisms are of prime importance, essentially similar in their action, and the one may replace the other. Both are undoubtedly active in normal respiration. Posterior root proprioceptive and cutaneous sensation cannot substitute for these mechanisms; and though they undoubtedly modify the respiratory rhythm, they cannot initiate it. Similarly, Stella (1939) has shown that the stimuli originating in the chemoreceptors of the carotid body, though increasing the tonic inspiration of apneusis, cannot initiate rhythmic respiration in the absence of vagal and pontine pneumotaxic mechanisms.

The vagal mechanism probably exerts its effect on the respiratory center in a reflex manner through afferents sensitive to lung stretch (Adrian, 1933). To account for the action of the pneumotaxic center is more difficult. It seems logical to us to postulate that the act of inspiration sets up impulses in ascending pathways to the pneumotaxic center, which through a closed neuron arc, lead to inspiratory inhibition in a manner analogous to the inhibition produced by the reflex stretch stimulation of vagal afferents (Pitts, Magoun and Ranson, 1939b). We are led to this view by the close similarity between vagal and pontine pneumotaxic influences. A second possibility, that the pontile center generates a spontaneous rhythm and is thus fundamentally dissimilar to the vagal mechanism, has at present absolutely no evidence to support it. A third possibility, that the pontine pneumotaxic center is activated reflexly by pro-

prioceptive afferents in a manner analogous to the reflex activation of the vagal mechanism, is definitely excluded by Stella (1938a). He found respiration rhythmic and relatively normal after section of the dorsal spinal roots from C_1 to T_{11}, section of the cord at T_{11}, and bilateral cold blocking of the vagus. Our own results are confirmative of this to the extent to which they have been carried. In figures 4C and 4D the medial lemniscus system has been sectioned with absolutely no effect on respiration, and the response to vagal block is identical to that before lemniscus section. To the extent that respiratory afferents are carried by the medial lemniscus, this is confirmative of Stella.

The experiments quoted in the introduction as providing evidence that the isolated respiratory center is spontaneously rhythmic, have two features in common; all eliminated the reflex vagal drive to the respiratory center, but all apparently left intact connections with the pneumotaxic center.

The other question, is the respiratory center spontaneously active, cannot be answered with certainty. However, if activity is spontaneous, such spontaneous activity is confined to the inspiratory center, i.e., tonic inspiration, not tonic expiration, results from removal of rhythmic drive. Stella (1938a) eliminated most afferent sensation by extensive posterior root section and cranial nerve section, and found that this combined with pontile decerebration did not prevent the development of apneusis on vagal block. This would seem to indicate that spontaneous activity within the inspiratory center leads to the development of apneusis. However, Pitts, Magoun and Ranson (1939b) have pointed out that the extensive synaptic connections demonstrated within the inspiratory center may be the basis of a self exciting closed neuron circuit, which might obviate the necessity for large scale (continuous) spontaneous activity. The nature or origin of the initial discharge would still remain unknown.

Marckwald originally described the occurrence of a type of regular slowly rhythmic inspiratory cramp (apneusis) which sometimes developed in pontine decerebrate animals after the initial prolonged cramp resulting from vagal section. We noted in a previous paper the appearance of this type of respiration in a few animals so prepared after prolonged artificial respiration. Marckwald's observation that it resulted only in animals in which the section spared the trigeminal nuclei and that it represented a poorly developed and not normally active pneumotaxic mechanism at a trigeminal level, is tentatively accepted in the absence of specific data on this question.

The polypnea and panting movements of a decorticate preparation have been shown to be mediated, in all probability, by the pontine pneumotaxic center and its efferent pathways connecting with the medullary respiratory center. Keller has previously stated that there is a region in the mes-

encephalon or upper pons which mediates panting and which is very sensitive to operative interference, and has shown that panting is mediated by pathways laterally placed in the pons (Keller, 1929, 1938). Polypneic influences ascending from spinal levels may operate in the absence of the pontile pneumotaxic center, but then require the activity of the vagal pneumotaxic mechanism to exert their usual effect. Sciatic stimulation during apneusis is able, however, either to increase or decrease inspiration.

The lateroventral site of the pathways connecting the pneumotaxic center in the pons with the medullary respiratory center is approximately that of the rubrospinal tracts; but the demonstration that the red nuclei lie cephalic to the pneumotaxic center destroys the significance of this correlation. Its position, if similar in man, would make it vulnerable in thrombosis of the posterior inferior cerebellar artery or in trigeminal tractotomy. However, the margin of safety is great in the cat, for even its bilateral interruption is not fatal so long as the vagi are intact.

It has been repeatedly observed that cold blocking of the vagi in an animal in which the only other operative interference has been to trephine the skull leads to respiration with the chest in a more inspiratory position. This change to an inspiratory mid-position is most marked in those animals which show a marked degree of decorticate panting. In these animals (see fig. 4) panting continues without much change in rate or amplitude, but the chest assumes a tonic inspiratory position. In animals which do not pant (fig. 3, B1, C1 and E1) the shift of the chest to an inspiratory position is equally evident though accompanied by increase in amplitude and decrease in rate. The significance of these tonic changes is not known.

SUMMARY

Rhythmicity of respiration results from the activity of two bilateral mechanisms: one, a brain stem pneumotaxic center; the other, a vagal reflex system sensitive to lung stretch. These two mechanisms are similar in action and mutually replaceable. A fraction of one of these two systems, active on one side only, is sufficient to impart rhythmicity to respiration. With both systems bilaterally and completely eliminated, a maintained tonic inspiration (apneusis) results.

The brain stem pneumotaxic center has been localized to the tegmentum of the upper few millimeters of the pons. The red nuclei and inferior colliculi are without functional significance in imparting rhythmicity to respiration. The pathways connecting the pneumotaxic center with the medullary respiratory center are located ventrally and far laterally in the pons and upper medulla, ventral to and slightly medial to the descending root of the trigeminal nerve.

Decorticate polypneic panting is probably mediated through the pneumotaxic center and its descending pathways by facilitation from some

higher mechanism released from cortical control. Reflex pain polypnea may be produced only in the presence of some functional rhythmic respiratory drive. When this drive is eliminated (apneusis), sensory stimulation leads only to slight decrease or increase of the tonic inspiration.

If spontaneous activity occurs within the respiratory center, it is limited to the inspiratory division. Spontaneous rhythmicity is not a property of the medullary respiratory center.

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SELECTIVE VASCULAR REACTION PATTERNS IN THE NASAL SEPTUM AND SKIN OF THE EXTREMITIES AND HEAD[1]

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Certain generalizations concerning vascular reactions in the skin have received general acceptance and are well supported by the vast literature on the vasomotor system. Thus, cutaneous vasoconstriction results from cold, painful stimuli, shock, fainting, mental effort, emotion, etc., while cutaneous vasodilatation results from local heat or from a rise in body temperature. It is generally believed that these reactions are shown more or less uniformly in all skin areas although the data supporting this idea have been obtained chiefly from observations on the limbs, hands, feet, fingers, toes and ears. It is not often that one encounters experiments on other skin areas. Much of the data on the limbs has failed to distinguish reactions in the skin from those occurring in deeper tissues.

There are, however, various experimental data which suggest that vascular reactions in the skin may show regional differences in degree and direction. Thus, there are striking differences in forearm and digital skin reactions to sensory stimuli, adrenin, cooling and warming the body (10). Vascular reactions to thermal changes show important differences in the forehead, fingers and toes (21), the toes constricting sooner with a fall in environmental temperature than do the fingers, the forehead vessels remaining open (9). There are large regional differences in the richness of the arterial supply (12). In only some skin areas is the arterial supply increased in warm weather (12). Reactions in the skin to histamine are most marked, as judged by the rise in skin temperature, in the cheek, neck and chest, and negligible over the arms and legs (23)—this difference may be purely qualitative and due to differences in cutaneous blood supply. Likewise, the patterns for sweating responses in normal persons to hyperthermia, emotion, pilocarpin and acetyl-beta-methyl choline suggest that vasomotor patterns may also exist (18).

This paper develops this suggestion further, showing the highly selective character of vascular activity in the skin and nasal septum, as it is mani-

[1] Preliminary reports. Proc. Am. Physiol. Soc. 335, 1937; 99, 1938; 477, 1939. Proc. Soc. Exper. Biol. and Med. **38:** 628, 1938.

fested in the so-called spontaneous waves, and in the reactions to psychic influences, a deep breath, the cold pressor test, and breath holding.

METHODS. The technique of photoelectric plethysmography of the skin and nasal septum as previously described (11, 12) was used in making the observations recorded below. Improvements in amplification techniques which are being reported elsewhere (13), have been incorporated in these studies. Mechanical plethysmography was used on the fingers and toes in a few instances. All subjects were apparently healthy young male medical students unless otherwise indicated. Most observations were made with the subject in a sitting position after a period of rest of twenty minutes or more. Room temperatures did not vary widely, although the laboratory is not air-conditioned, being usually about 25.0°C. No attempt was made to secure quantitative data. However, the qualitative differences in reactions are roughly comparable in extent. The experimental material has accumulated over a period of three years during some 80-odd observation periods. By the latter we mean all of the observations made on a subject at a single sitting. Some subjects served a number of times, others only once. The number of observations on spontaneous waves is considerable since they are routinely made during "control" periods for other studies.

Spontaneous waves. So-called spontaneous waves in plethysmograms of the limbs have been reported by many observers. They have received detailed classification into waves of the first, second, third and fourth orders. Distinction has been made between waves due to purely peripheral factors and those arising in changes in vasomotor tone (22). Details on their occurrence in the arm are given by Uhlenbruck (22). Maximal thermal dilatation seems to diminish and even eliminate them in the ears and digits. They are commonly seen when environmental temperatures are in the range of vasomotor regulation of body temperatures. These waves have been interpreted as due to mass rhythmic activity of the vasomotor system in the regulation of heat losses through the variations in digital blood flow (5). Although this interpretation of a vasomotor origin of these waves probably applied to some of our data, it is yet possible that other waves are purely local in character, involving changes in vessel tone independent of the innervation. Still other waves may be the result of limited and selective vasomotor activity.

Simultaneous plethysmograms of the fingers of the same hand, of the fingers of both hands, and of the palmar and dorsal surfaces of the hands, show that the spontaneous waves are almost always synchronous in the skin of both hands. One will also see minor waves in one finger pad and not in others, but such instances are not encountered frequently. The spontaneous waves are often completely synchronous in the fingers and toes, although one will more frequently see differences in this combination.

The synchronicity of these waves would imply a vasomotor origin. We so interpret them.

In the case of Raynaud's phenomena, however, independent activity of the vessels of the hand may be strikingly evident. That sympathectomy need not abolish these waves is shown in the comparisons in which the denervated vessels are more active than those still innervated. Since similar independent activity differing in degree is sometimes seen in normal subjects one may wonder if the spasms of Raynaud's phenomena are not an exaggeration of normal behavior due to the "local fault" in the vessels suggested by Lewis (16).

If the spontaneous waves in the fingers and toes have a vasomotor origin, one might then possibly expect waves occurring in other areas to synchronize with those in the digits, although it is becoming increasingly evident that the circulation in the digits is an exceedingly sensitive indicator of vasomotor activity. We have had the frequent opportunity to make comparisons between digit waves and waves in other areas during our exploration of the latter.

The spontaneous waves which we have seen may be classified into two groups: 1, those synchronous with respiration and due apparently to mechanical effects of breathing (19)—we are not concerned with these waves in this paper; 2, waves longer than the respiratory cycle, varying from ten to sixty seconds or more in length.

Waves of the second group may or may not appear synchronously in several areas. They may be limited to the fingers (fig. 1A) or the forehead (fig. 1B, C, D) or the ear (fig. 1E, F) or the nasal septum (fig. 2B), without concomitant waves in the other areas being observed at the same time. When occurring simultaneously in several areas, they may or may not be opposite in direction (fig. 2A) and they may differ in period. They may be synchronous and similar in the three areas, finger, ear, side of nose, and of longer period in the nasal septum. Rarely do the waves synchronize in all areas, but this has been seen for the combination: finger, ear, side of nose, nasal septum.

It is more usual for waves in head skin to be independent of those in the digits with respect to both timing and direction. Waves may appear in the forehead (fig. 1A, B, C, D) and ear (fig. 2A, 2E) and be absent from the finger. They may be more marked in either forehead or ear and fail to synchronize. In the case of forehead waves being opposite to but synchronous with finger waves, it was thought that this might be due to "passive" changes in the forehead as a result of blood pressure changes. Attempts to make the correlation by continuous recording of directional changes in brachial blood pressure, failed to show it. Yet it is worth noting that head skin waves are usually dilatations while finger and toe

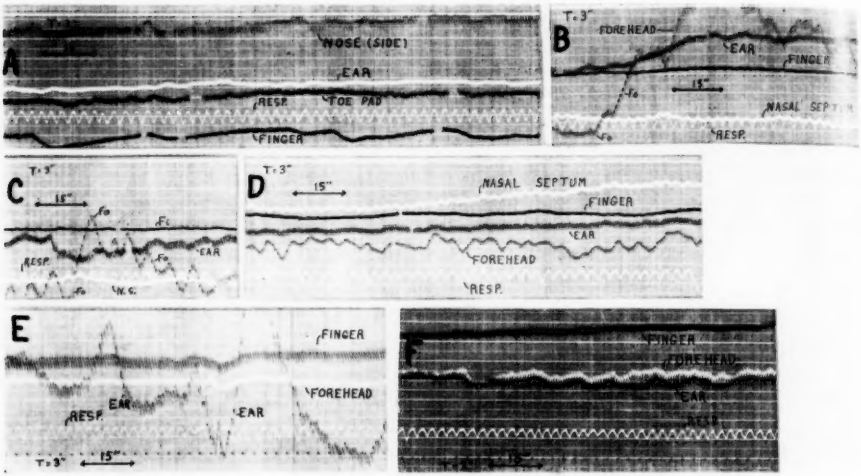

Fig. 1. Spontaneous waves: A. Finger tip, toe pad, ear, side of nose. Normal male. Room temperature 27.0°C. Waves only in fingers. B, C, D. Segments from continuous record of case of hypertension. Male, age 30. Ear, forehead, nasal septum and finger. Note waves in forehead becoming smaller. E, F. Segments one hour apart from records on a case of hyper-sensitive carotid sinus. Finger, ear, forehead. Note disappearance of waves in ear.

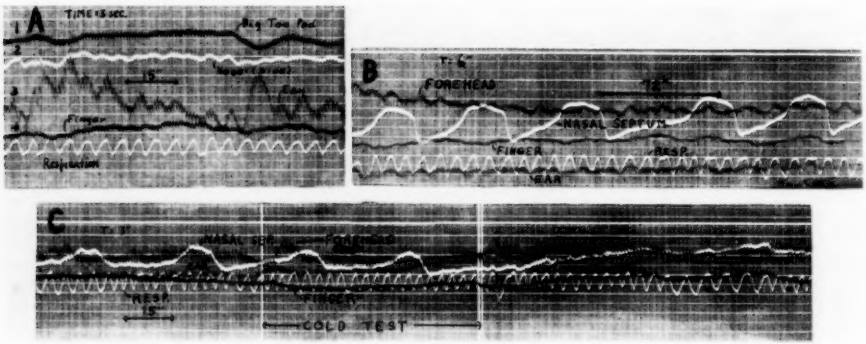

Fig. 2. Spontaneous waves: A. Big toe pad, finger, side of nose, ear. Normal male. Room temperature 26.0°C. B. Nasal septum, forehead, ear, finger. Normal male. Room temperature 25.0°C. C. Nasal septum, ear, forehead, finger. Normal male. Room temperature 25.0°C.

waves are usually constrictions. The ear waves are more varied in character as are also those of the nasal septum.

Waves in the ears, nasal septum (fig. 2B) and fingers may make their appearance or become more marked following the cold pressor test possibly as a result of the vasomotor disturbances attendant on this test.

Waves in head skin and ear may seem occasionally to bear a definite relationship to respiration, but each wave may occupy two or three respiratory cycles. Vascular disturbances as with amyl nitrite will augment these waves but leave their relation to respiration unchanged.

Some of these phenomena are illustrated in figure 2. The contrasting activity of ear and toe (fig. 2A) with relative inactivity of finger and side of nose show not only in the changes in blood content but also in the amplitudes of the volume pulses. The ear dilates at the same time that the toe constricts. Toe activity was more pronounced prior to this record, the reverse being true for the ear. If the change in the ear were "passive," the volume pulse would tend to remain constant. Since it increases in amplitude in the ear at the same time that it decreases in the toe, one may deduce that an "active" dilatation occurred in the ear.[2] The record may be considered as an example of reciprocal vasomotor effects.

The extreme waves in the nasal septum in Fig. 2B are difficult to interpret. They have been seen several times, but are unusual. They first appeared during a cold pressor test, disappeared for several minutes and then continued until the end of observation one-half hour later. Other vascular areas under observation were inactive and showed nothing like these waves at any time. The septum waves were unaffected by respiratory disturbances such as coughing. They had a variable duration of 30 to 70 seconds. They continued apparently unaffected by the administration of amyl nitrite which effectively dilated the forehead and ear without noticeable effect on the septum (a most unusual case). Their onset was marked by constriction as indicated by the decreased amplitude of the volume pulse and later by congestion as indicated by a rapidly rising blood content. Neither the constriction nor the congestion disappeared during the observation period.

A somewhat similar case is shown in figure 2C. In this instance, the waves were present in the septum when observation began but disappeared at the conclusion of the cold pressor test to reappear later. Spontaneous waves have likewise developed occasionally in the forehead and ear without corresponding waves in the finger and nasal septum on the administration of amyl nitrite or of the cold pressor test.

Psychic and auditory stimuli. Since most of our subjects have been medical students or younger members of the faculty, it seems probable that psychic influences have been active in many of our observations. It is extremely difficult for an intelligent mentally active person to remain "passive" during a prolonged observation. Varying interest in the experiment or

[2] The argument that the size of the volume pulse is a measure of arterial flow has been discussed in a previous paper (12). Burton and Taylor (5) have similarly used the changes in volume pulse amplitude as a measure of arterial reactions in the fingers. They find a good correlation between blood flow and volume pulse amplitude. The effect of the cardiac stroke volume must be kept in mind.

thoughts concerning other matters may be factors in the genesis of "spontaneous" waves, particularly in the fingers which seem to be exquisite indicators of vasomotor activity not sufficient in extent to show itself in blood pressure changes. The authors serving as subjects have watched for such relations but one is easily deceived in the attempt. We have, therefore, had recourse to instances in which suitable psychic stimuli were supplied to students. This was easy to do since they were students in the department's courses. Often, however, the resulting excitement produced movements which created artifacts in the records.

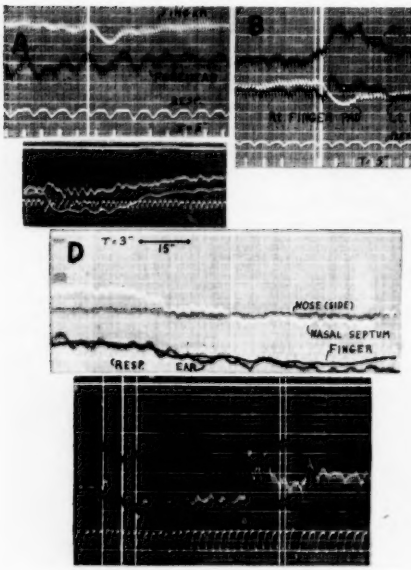

Fig. 3

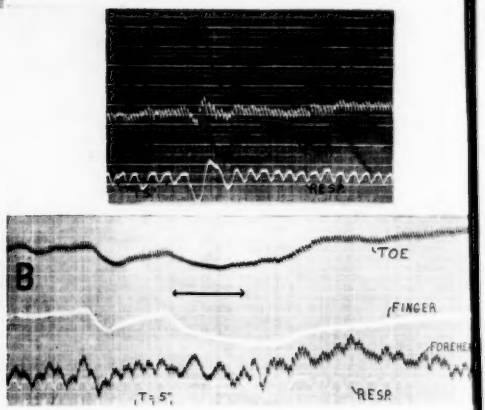

Fig. 4

Fig. 3. Cutaneous vascular responses to loud noises (A and B) and psychic stimuli (C, D, E). Description in text.

Fig. 4. Cutaneous vascular reactions to (A) a deep inspiration and (B) to a breath hold. Description in text.

Auditory stimuli may be partly responsible for the effects of the psychic stimuli. Responses to loud noises are illustrated in figure 3A, B. Finger constriction is always obtained. The extent varies as much with the element of surprise as with the loudness of the noise. Head skin responses are variable: either dilatation (fig. 3B) or no change (fig. 3A). The dilatation when it occurs is active since it involves increased amplitude of the volume pulse as well as increased blood volume in the area. The nasal septum tends to follow the finger although it may show no change. The ear may show constriction or fail to respond.

The usual response to a psychic stimulus ("Why did you fail?") shows in figure 3C: finger constriction, no change in head skin. Instruction on what to expect in the action of a drug (amyl nitrite) which was to be administered later, produced a prolonged constriction in finger, ear and nasal septum without effect on the skin of the nose (fig. 3D). Instruction on the cold pressor test (fig. 3E—first three signals), yielded constriction in the finger. Ear and forehead responses cannot be considered as constrictions since the volume pulses are unchanged, even though the blood content decreased slightly. The opposite hand was then immersed in water at body temperature (last two signals) when the subject was expecting the pain resulting from ice-water. The finger promptly constricted. The changes in the ear and forehead seem to be due to mechanical factors resulting from the deep breath which the subject made. (Compare below with the responses to a deep breath.)

Effects of a deep breath. It was first shown by Binet and Sollier (3) and later by Uhlenbruck (22) that a deep breath is followed by a temporary vasoconstriction. This vasoconstriction in the fingers and toes depends on the integrity of the vasomotor innervation, being no longer obtained after denervation by sympathectomy or other interruption of vasomotor fibers (4). Others (8) in a study of digital and brachial arterial blood pressures continuously recorded, find a rise in digital arterial pressure (due to digital constriction) but a fall or no change in brachial blood pressure. Since the digital constriction may proceed to obliteration of the volume pulse without change in general arterial pressure, it seems improbable that the vasoconstrictor response to a deep breath is general.

Deep breaths are made involuntarily at various intervals by the resting subject. There is, therefore, the frequent opportunity to observe the responses without the complication of other stimuli. Figure 4A shows typical results in the three cutaneous areas, finger, forehead and ear: constriction in the finger (decreased blood content and volume pulse); the suggestion of a slight dilatation in the forehead (small fleeting increases in volume pulse and blood content), and a diphasic response in the ear which seems to mimic that in the finger. It is difficult to interpret the ear response. The onset in the decrease in blood content occurs at the same moment as that in the finger, suggesting a constriction, but the changes in the volume pulse are slight compared with those in the finger. Since changes in blood content result also from changes in venous pressure, we pay more attention to the volume pulse as a criterion of the arterial reactions. On this basis, the constriction in the ear is very slight and very brief in duration. The ear responses to a deep breath are more like those in head skin than in the fingers. The effects on head skin seem to be due to mechanical factors resulting from the large excursions in intrathoracic pressure. We see no evidence for a true dilator response here.

Interesting modifications have been observed in these responses in some

pathological subjects. Thus, in a case of hypersensitive carotid sinus, the finger responses seemed prolonged, and some very obvious constrictions occurred in the ear, the forehead responses being unchanged. In a case of Raynaud's phenomena with a unilateral cervical sympathectomy, the finger responses were abolished on the operated side as had been shown previously (4) and the forehead and ear changes were still similar to those in the normal subject. The ear responses on the unoperated side were peculiar, however; a marked constriction similar to that in the finger (the response here was typical) followed by a very marked and prolonged dilata-

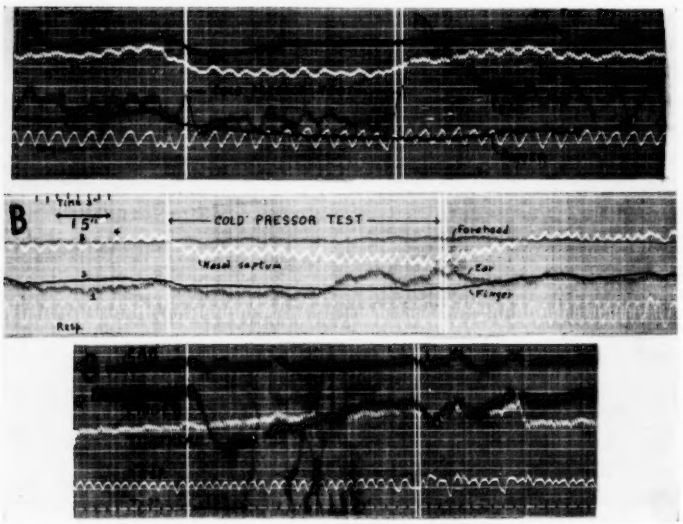

Fig. 5. Cutaneous vascular reactions to the cold pressor test. A and B, normal subjects. C, hypersensitive carotid sinus. Immersion of hand between vertical signals.

tion. The forehead response was of the usual type. The responses in cases of hypertension presented no unusual features.

The cold pressor test. Cold has been frequently used as a stimulus to elicit vasomotor reactions. Constriction in the skin (of the extremities) and respiratory passages is regularly observed. This stimulus has been standardized by Hines and Brown (15) for the measurement of vasomotor reactivity as indicated by the changes in arterial blood pressure. This points to a far-flung vasomotor response, the intensity and possibly also the distribution of which may vary considerably in different individuals. We have been interested in examining the vascular responses in the skin and nasal septum not only in relation to their selectivity but also with

respect to the classification of the subjects as hyper-reactors in the sense that Hines and Brown use the term. Although the subjects studied have been normal individuals as well as some cases of hypertension, they are not sufficiently numerous to permit statistical correlation with the classification of the blood pressure responses used by Hines and Brown. The test has been run following the standard conditions of Hines and Brown.

The usual responses (fig. 5) are: constriction in the finger and toe (fig. 5A) in all cases; and, also, in the nasal septum (fig. 5B). The constriction in the finger and nasal septum is usually maintained during the entire period of immersion of the opposite hand. That in the toe is initially greatest and often tends to disappear (fig. 5A) during the immersion. The constriction in the finger tends to be complete enough to obliterate the volume pulse. We have seen no qualitative differences in this respect between cases of hypertension and normal subjects. The reactions in the toe and nasal septum are not as marked as those in the finger. The ear responses (fig. 5A, B, C) are more variable: occasionally, a slight but definite constriction (fig. 5A); more frequently, either no change (fig. 5B and C) or a later dilatation. These changes have borne no relation to whether the subject is a hyper-reactor or not. The forehead skin shows either no change (fig. 5B and C) or an increased blood content which may be either passive (simple venous congestion) or active (increased volume pulse). Thermo-couple readings on forehead skin agreed with this interpretation of the plethysmograms. Fewer observations on the side of the nose agreed in general with those on forehead skin. An exception (fig. 5A) showed an active constriction (decreased blood content and volume pulse)—this subject shows the usual responses in the forehead. Cases of hypertension showed similar responses in the forehead.

The cutaneous vascular reactions to the cold pressor test in a case of hypersensitive carotid sinus (fig. 5C) who is not an hyper-reactor were interesting in view of the modification of pressor responses by the carotid sinus. The plethysmograms presented nothing unusual except for the rapid disappearance of the finger constriction during the immersion of the opposite hand. The record shows that the volume pulse has increased again to the pre-immersion level within 30 seconds after immersion began although finger volume recovers more gradually. Relaxation of the finger arteries late during the immersion is encountered infrequently. This is the most striking example, but it is uncertain that it is related to the hypersensitive carotid sinus.

Breath holding. Voluntary breath holding has been offered as a substitute for the cold pressor test (1). Since the stimulus here probably arises centrally instead of in peripheral sense organs, we have been interested in comparing the cutaneous vasculár responses. Although the number of subjects is small, they are the same as those on whom the re-

sponses to the cold pressor test have been recorded. The results have been the same (fig. 4B): progressive constriction in the finger to almost complete obliteration of the volume pulse, less complete constriction in the toe, no changes in the ear or forehead which could not be accounted for by variations in intra-thoracic pressure. The finger constrictions often begin when the expiration, which is to precede the breath hold, is made, or they may not appear until some discomfort is experienced.

Discussion. Rhythmic activity in the smooth muscle of blood vessels has been seen in several areas, e.g., ear (6, 20) pia (14), limbs, spleen (2) as well as in isolated surviving vessels. Such rhythmic activity may be independent of nervous influences (2, 6) or it may be exactly correlated with vasomotor activity (24). Hence, the interpretation of data illustrated above may use the possibility of rhythmic changes in the muscle tone of vessels independent of the innervation, or the possibility of patterns in vasomotor activity, highly selective with respect to the vascular geography and the direction of the change, as the cause of the waves. Decision as to which of these two possibilities is operative in a specific instance is not always easy. The fact that the waves in hand skin usually occur synchronously and similarly in the entire cutaneous circulation here argues for the need of a vasomotor coördination of the vessels to explain such waves. Burton and Taylor (5) have so interpreted their origin. Yet even in normal subjects one will see dissociation in the activity of the vessels of neighboring fingers (the phenomenon is greatly exaggerated in Raynaud's disease), a fact which is difficult to interpret on a vasomotor basis. (There is the additional possibility that an axon reflex may account for dissociation in the finger waves in the sense that Lewis (17) used the concept to explain asynchronous oscillations in the temperature of supercooled neighboring fingers.) It would seem, therefore, that both possibilities are available in the hand circulation and that the waves of vasomotor origin are ordinarily dominant in the healthy subject.

When one attempts to extend this interpretation to waves occurring in other skin areas (as head skin) simultaneously observed, certain facts must be considered. Waves in head skin are usually independent of hand skin waves in timing, direction and extent. They may appear when there are no waves in hand skin. The converse is also true. To interpret these waves as having a vasomotor origin would necessitate a high degree of selectivity in cutaneous vasomotor patterns. We are inclined to this explanation since cutaneous vascular reactions to various procedures support this thesis.

We cannot harmonize the independence of head skin waves from hand skin waves with their origin in mass discharges of the sympathetic system such as those involved in Traube-Hering waves or in the rhythmic fluctuations in finger flow observed and attributed by Burton and Taylor (5) to mass effects of the vasomotors.

Since the observations on which this paper is based are on skin areas in some of which arterio-venous anastomoses are present (7), the relation of these vessels to these waves must be kept in mind. The techniques used do not permit the specific study of these vessels. It is possible, however, that some of the instances of independence in waves in the neighboring fingers may be an expression of arterio-venous anastomosis activity.

It seems doubtful that these waves serve any useful purpose. Rather, we are inclined to consider them as a normal over-shooting in the regulation of the skin circulation, particularly in relation to the regulation of body temperature as Burton and Taylor (5) have suggested. The greater lability of the finger circulation correlates with the greater degree of spontaneous activity here. The relative lack of response of forehead skin vessels to climatic changes (12) correlates with the smaller degree of spontaneous activity there. Yet, one will see very pronounced waves in head skin. They have been so marked here in several cases of essential hypertension that one wonders if they are then an expression of vasomotor instability showing itself at the moment in vascular changes in the head skin. Such cases would seem to be examples of selective vasomotor patterns in the skin.

Both auditory and psychic stimuli are well-known means for producing generalized vasomotor responses sufficiently extensive and intense to raise the arterial blood pressure. It is, therefore, of interest to note the absence of vasoconstrictor effects in head skin (excepting the ear). In contrast, the more exposed and apparently more labile areas, the fingers, toes and, to a less extent, the ears, participate vigorously in the reaction and show it even when the blood pressure changes are negligible. The tendency for head skin vessels to dilate during these vasoconstrictor reactions may be due to a rising blood pressure overcoming a weak constrictor effect here (analogous to the cerebral circulation), but at times the increases in the volume pulse are very suggestive of a vasodilator reflex in this area. We await the opportunity to compare the responses after nerve block and denervation. The cutaneous vascular reaction patterns in response to these stimuli show a general similarity with those to pain, intense cold, and breath holding.

Since the finger and toe reactions to a deep breath have been clearly demonstrated (14) to be due to the vasoconstrictor innervation, it is obvious from the data and from the changes in brachial blood pressure (8) that only very limited vascular areas participate. Certainly nothing like the finger reactions is seen in the head skin. Nor does one see here clean-cut evidence for an "active" dilatation following a deep breath. It is, therefore, surprising in view of the profound constriction occurring in the finger that the reaction is so limited in the area involved. It would, therefore, seem to be an example of a cutaneous vasomotor reaction highly selective with respect to its geography, although capable of great intensity.

The cold pressor test may be considered simply as a convenient means of supplying painful stimuli. It is obvious to any one using the test that the amount of pain experienced varies greatly with different subjects. The stimulus cannot be considered as standard or constant—it is possible that the breath holding test advocated by Ayman and Goldshine (1) offers advantages. Nevertheless, the pattern of the cutaneous vascular response has been very uniform; certainly much less variable than the blood pressure changes. Finger and toe constrictions occur almost always, while head skin tends to dilate. The pattern was the same in the cases of essential hypertension studied. This may mean simply that any stimulus applied to the surface of the body sufficient to excite vasoconstrictor reflexes will do so in the digits even though no widespread responses, as indicated in the blood pressure levels, occur. It had been thought that there would be considerable variation in the intensity of the digit constriction in accordance with whether the subject was classified as an hyper-reactor or not but the constriction is usually fairly complete (decrease in volume pulse almost to obliteration) in all cases. It could not be correlated with the degree of pain. The stimulus seems to be almost always sufficiently effective to elicit a powerful finger constriction. The absence of a response at the same time in the forehead and ear further indicates the selective geography of the vasomotor reaction involved in both the cold pressor and breath holding tests (1). The blood pressure changes may be expected when the vascular bed involved (e.g., splanchnic) becomes sufficiently large. Our data illustrate the value of the finger as a superior and exquisitely sensitive indicator of vasomotor reflexes which may or may not show elsewhere or in blood-pressure changes.

The absence of vaso-constrictor reflexes in the skin of the forehead reminds one of the difficulties experienced in obtaining them in the cerebral circulation. The two vascular beds are analogous in their stability, the constancy of their blood supplies, and their relative freedom from vasoconstrictor influences. The vessels in both areas are dilated readily by the same dilator drugs and passively by a rise in arterial blood pressure. They also seem to respond similarly in histamine headache. These similarities suggest the possibility of using forehead skin plethysmograms as an indicator of the cerebral circulation, a point which calls for a more complete exploration than can be detailed in this paper.

SUMMARY AND CONCLUSIONS

The photoelectric plethysmograph previously described (11, 12) has been used to study the vascular activity in the skin of the head and extremities and of the nasal septum of the resting normal man.

Spontaneous waves may appear, either as constrictions or dilatations,

usually as constrictions in the extremities and dilatations in head skin but of varied character in the ear and nasal septum. They may be limited to one area or they may appear in several or in all areas being observed. They are more frequently seen in the fingers but may be just as marked in other areas. These waves may or may not synchronize in the different vascular areas and they may or may not differ in direction and extent.

Some of these waves appear to be due to local activity of smooth muscle independent of the innervation. The majority of the waves seem to be due to vasomotor activity and possibly represent overshooting in vasomotor regulation.

Auditory and psychic stimuli, deep breaths, the cold pressor test and breath-holding all yield marked vasoconstrictor effects in the digits and toes, in hand and foot skin, and in the nasal septum, but variable effects in the ear and head skin. The ear will often constrict but it is unusual to obtain a constrictor response in the forehead where either "active" or "passive" dilatation or no change results from vasoconstrictor stimuli.

These responses and the character, distribution and timing of the spontaneous waves support the concept of selective vasomotor patterns in the circulation of the skin and nasal septum. These patterns are selective with respect to the geography, timing, direction and extent of the vasomotor activity in these vascular areas.

The similarity in the direction and character of the vascular changes in the forehead skin with those known to occur in the cerebral circulation suggests the possibility of using forehead skin plethysmograms as an indicator of the cerebral circulation.

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THE GLUCOSE AND LACTIC ACID EXCHANGES DURING HYPOGLYCEMIA[1]

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The wide use of insulin has made it important to investigate the physiological changes occurring during hypoglycemia, especially since prolonged hypoglycemia is used as a therapeutic measure in the treatment of schizophrenia. A study of the carbohydrate balance of the brain has revealed that the amount of glucose absorbed by that organ is diminished during hypoglycemia (1). It still remains to determine the carbohydrate balance of the other organs during this condition.

METHOD. Dogs under nembutal anesthesia were used for these studies. Twenty units of insulin were injected each hour until the desired degree of hypoglycemia was attained. Blood samples both before and after the injection of insulin were collected practically simultaneously from the hepatic vein, portal vein, femoral vein, and femoral artery. This blood was analyzed for glucose and lactic acid by the methods of Hagedorn and Jensen (2) and Friedemann, Cotonia and Shaffer (3) respectively. By comparing the contents of the arterial and venous blood samples, the glucose and lactic acid balance of liver, muscle, and the intestine could be determined. Differences of 5 mgm. per cent or more were considered significant. In determining the carbohydrate balance of the liver, the portal blood was weighted 3 times that of the arterial.

RESULTS. Twenty-six observations were made on 7 dogs (table 1). In the following analysis of the results a value of 54 mgm. per cent was taken as the upper limit of hypoglycemia and the lowest was 7 mgm. per cent representing unfermentable reducing substances. The exchanges of the liver reveal that in 15 of 17 instances that organ added significant amounts of glucose to the blood and in no case was sugar absorbed. In fact in most instances hypoglycemia increased the output. This is in accordance with the results of Crandall and Cherry (4). The concentration of lactic acid in 12 of 16 observations exhibited no significant change. In one instance lactic acid was added and 3 times removed. It should be noted that in 2 of these 3 observations the lactic acid level of the blood was

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high because of the intravenous injection of the substance. The intestine absorbed glucose in 11 of 18 instances and in no case added that substrate

TABLE 1

Glucose and lactic acid exchanges of liver, intestine and muscle, milligram per cent

DATE OF EXPERIMENT	ARTERIAL BLOOD SUGAR	ARTERIAL LACTIC ACID	LIVER $\frac{3P + A}{4} - H$		INTESTINE A-P		MUSCLE A-V	
			B.S.	L.A.	B.S.	L.A.	Sugar	Lactic acid
10/ 6/38	86	16	+4	+8	−8	−9	−10	−6
	18	15	+17	−5	−9	−2	+10	−7
	101	9	+12	−3	+12	+7	−23	+11
	52	20	+14	−2	−26	−8	−33	−7
	29	*85	+4	−13	0	+14	+2	+2
10/11/38	75	11	+9	0	−1	+1	+2	+1
	9		+7		+1		+1	
	79	19	+2	−8	+2	+7	−7	+4
	22	15	+13	−2	−5	+1	−2	+3
	12	19	+7	0	−1	−6	−2	+3
	10	*135	+6	+3	+1	−15	−3	−13
Portal blood								
10/17/38	81	27	−1	Clotted	+11		0	0
	48	25	+12	+6	−12	+1	−9	−6
	31	38	+8	−3	−9	0	−7	−1
	52	33	+1	0	−2	0	−5	+1
	43	*140	+11	−11	−9	+28	−9	+12
10/20/38	88	15	+6	+4	−14	−2	−2	+2
	29	15	+24	−2	−13	+2	−11	+6
	54	30	+11	−4	−11	0	−15	+1
	20	32	+32	+4	−3	−3	−3	+3
10/31/38	79	31	+16	+8	−16	−1	−14	−5
	7	11	+25	+3	−5	+3	−4	+7
	14	39	+23	0	−2	+3	−4	+8
11/ 3/38	146	24	−13	−3	+9	−2	0	0
	47	14	+8	+2	−11	−1	−6	+2
12/16/38	51	22	+31	−11	−17	+9	−13	+1

\+ Added to blood.

− Absorbed from blood.

* Sodium lactate intravenously.

to the blood. The lactic acid exchanges of the intestine were not significant in 11 of 17 observations. Three times lactic acid was removed and

3 times it was poured in the blood stream. In two of these instances the lactic level of the blood was greatly raised. In 9 of 18 observations muscle absorbed glucose, and in one case only was that substance added to the blood. The lactic acid exchanges of muscle revealed no definite trend since in 9 of 17 experiments the differences were not significant. Four times each lactic acid was added and removed, in an equal number of observations. However, it should be noted that in 2 of 4 cases when lactic acid was added this occurred with a high level of this substance in the blood.

Discussion. These results reveal that during hypoglycemia the liver continually adds glucose to the blood and this glucose is removed by muscle and intestine except when the concentration of fermentable glucose becomes negligible. Under the conditions of our experiments the lactic acid exchanges of the various organs were not significant. Thus, during hypoglycemia the small amount of sugar present in the blood is maintained by its constant release from the liver. This observation may be contrasted with that of the hepatectomized animal which soon succumbs unless glucose is administered (5). The results obtained with lactic acid are different from those previously observed, in which a definite cycle had been established. Lactic acid liberated by the muscle was removed by the liver (6) (7) (8). While this investigation was in progress Cherry and Crandall (9) emphasized the non-existence of the glucose lactic acid cycle when the level of the lactic acid of the blood was low. The concentration of the lactic acid of the blood was above 20 mgm. per cent in 13 of the present observations, an increased concentration of lactic acid which may be attributed in part to the effect of barbiturate in interfering with the recovery processes in muscle (10). Despite the high level of lactic acid, in only 3 instances was that substance removed by the liver. In an attempt to explain the inability of the liver to absorb lactic acid a suggestion made by Doctor Crandall (personal communication) should be taken into consideration, namely, that the conditions necessary for the function of the glucose lactic acid cycle require not only a high concentration of lactic acid but also a period of fasting probably necessary to reduce the glycogen content of the liver. In the present investigation two animals had been fed on the day previous to the experiment while in our earlier work in which the glucose lactic acid cycle was demonstrated, the dogs had been fasted on the average for 1 week. This factor may explain the absence of the glucose lactic acid cycle in the present experiment. Since in these experiments lactic acid cannot be regarded as the source of the hepatic glucose, the origin of the sugar liberated in the blood stream may be attributed partially to the glycogen still present in the liver (11) and also to gluconeogenesis from proteins. The present results reveal the hemostatic mechanism for the maintenance of life, for the liver continues to

supply glucose for use by other organs of the body even during prolonged hypoglycemia.

CONCLUSIONS

The glucose and lactic acid exchange of liver, intestine, and muscle were studied during hypoglycemia. Twenty-six observations were made on 7 nembutalized dogs in the post absorptive condition. During hypoglycemia the liver is continually supplying the blood with glucose, and the lactic acid exchanges of the various organs are negligible.

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BORON IN ANIMAL NUTRITION[1]

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Probably the first demonstration of the presence of boron in animal tissues and animal products was made by Bertrand and Agulhon (1, 2, 3). By the use of the turmeric paper coloration method these workers found that all animal organs contained at least traces of this element and that milks of all kinds contained relatively high concentrations. Boron was found to be present in both egg yolk and egg white, although the concentration in the white was about ten times higher than in the yolk.

The presence of boron in milk has been confirmed spectrographically by Wright and Papisch (4), Blumberg and Rask (5) and Drea (6), and in egg yolk by Drea (7). But contrary to the findings of Betrand and Agulhon, no boron could be detected by Drea in egg white by this method.

Very few attempts have been made to relate the boron concentration in animal tissues and products to the amount of boron in the diet. Pharmacological interest has led to a considerable amount of work on the rate of excretion of boric acid and other boron compounds after feeding. The results obtained by practically all workers (Michaelis and Maass, 8; Presnell and Brill, 9) have shown that boron compounds are eliminated with extreme rapidity. Wiley (10) detected the presence of boron in the urine, perspiration, feces and milk of humans after feeding five grams of boric acid. These materials were said to contain no boron naturally.

As far as we are aware there is no record in the literature of an attempt to determine whether or not boron is an essential nutrient for animals. Since it has been known for many years that this element is required by plants for normal growth and development we have thought it worth while to investigate its bearing on the nutrition of the rat. Our results on this problem are recorded in this paper, along with other studies on the biochemistry of boron.

EXPERIMENTAL. *The method for boron determination.* Smith (11) has described a color reaction of boron with quinalizarin, and has investigated

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some of the factors affecting the intensity of the color. We have applied this reaction to the determination of boron in biological materials, using the Evelyn photoelectric colorimeter. The ashing conditions and the method of separation of boron from the ash which we have used are essentially the same as those described by McHargue and Calfee (12), with the exception that the ash was taken up in phosphoric acid instead of citric acid. After the distillate is brought to dryness it is taken up in 2 cc. of glass distilled water and a 1 cc. aliquot pipetted into a standard colorimeter tube. Ten cubic centimeters of concentrated sulfuric acid are added slowly,

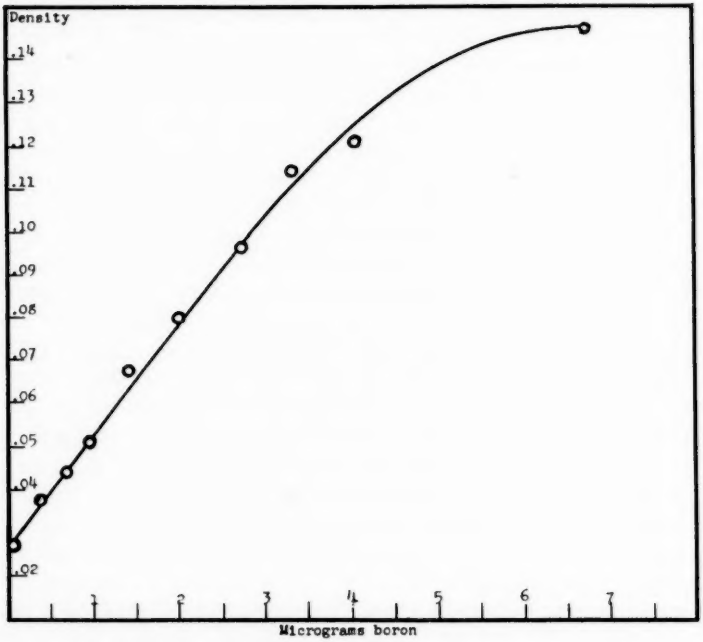

Fig. 1. Boron quinalizarin photoelectric colorimeter curve.

mixed, and cooled to room temperature. To the tube is now added 0.5 cc. of a 0.01 per cent solution of quinalizarin in 90 per cent (by volume) sulfuric acid. The tube is well stoppered and read on the colorimeter 4 to 12 hours later, at which time the color change will have reached a maximum and constant value. The 6,200 Å filter is used.

The standard curve shown in figure 1 is obtained by plotting the log values (densities) of the colorimeter readings against the corresponding known quantities of boron. The known quantities of boron are treated exactly as unknown samples. This curve cannot be made by using pure

solutions of boric acid without distillation, for comparison of the curves obtained by the use of distilled and non-distilled quantities of boron shows a rather constant 17 to 20 per cent loss on distillation. This loss occurs primarily in taking the distillate to dryness. For this reason this step should be as standardized as possible. The size of the sample is adjusted so that the final distillate will contain between 1 and 10 micrograms of boron, or 0.5 to 5 micrograms in the aliquot. Since the blank on the reagents is relatively large no value below 0.5 microgram in the 1 cc. aliquot is considered significant.

In reading the color intensity of the samples the galvonometer deflection is set at 100 on the scale using a tube containing the reagents only, i.e., the 10 cc. of sulfuric acid, 1 cc. of redistilled water and the 0.5 cc. of the dye solution. This automatically corrects for any slight variation in the density of the sulfuric acid. The final density of the solution has a pro-

TABLE 1

The effect of the final density on color intensity

Ten cubic centimeters of concentrated sulfuric acid, 0.5 cc. of dye and 1 cc. of a water solution of boric acid (equivalent to 5.3 micrograms of boron).

WATER IN EXCESS OF CUSTOMARY 1 CC.	APPARENT BORON CONTENT	PER CENT DIMINUTION OF BORON CONTENT
	micrograms	
0	5.30	0
0.1 cc.	3.40	17.0
0.2	2.75	48.1
0.3	2.08	61.3
0.4	1.60	70.0
1.0	Almost colorless	

found effect on the color intensity. This is shown in table 1. As little as 0.1 cc. of surplus water in the 11.5 cc. total volume causes more than a 15 per cent decrease in the apparent boron content. It is obvious from these results that extreme care must be taken in pipetting.

Boron free soft glass and platinum are used throughout. The percentage recoveries of boron added to milk samples are shown in table 2.

The boron content of milks. The boron content of the milks from a number of cows of various breeds was determined by the method just described. The results are shown in table 3. The milk was collected directly into a soft glass bottle from the right fore quarter at the beginning of milking. The determination on each milk was run in triplicate using 25 cc. samples. The average percentage variation between the three values obtained for each milk gives a measure of the reproducibility of the method and these average variations are shown in the last column of table 3. This series was run in late summer within the space of ten days so as to eliminate

TABLE 2

Recoveries of boron added to milk samples

SAMPLE (MILK)	BORON ADDED	BORON FOUND	BORON RECOVERED	RECOVERY
cc.	*micrograms*	*micrograms*	*micrograms*	*per cent*
25	0	2.60		
25	0	2.66		
25	1.36	4.00	1.37	100
25	1.36	4.08	1.45	107
25	2.04	4.70	2.07	101
25	2.04	4.60	1.97	96.5
25	2.72	5.10	2.47	90.8
25	3.40	5.78	3.15	92.5

TABLE 3

The boron content of normal milks

BREED	COW	PERIOD OF LACTATION	BORON PER LITER	AVERAGE PERCENTAGE DEVIATION BETWEEN TRIPLICATES
		months	*mgm.*	
Holstein	U-58	3	0.180	8.7
	65	4	0.138	6.7
	28	8	0.155	2.3
	34	8	0.199	4.2
	70	8	0.166	7.5
	44	12	0.145	5.6
	67	12	0.133	6.8
Brown Swiss	U-227	1	0.170	6.8
	822	4	0.185	2.0
	807	6	0.161	7.9
	815	7	0.184	1.3
	813	7	0.176	2.5
	808	9	0.220	6.7
Jersey	U-608	1	0.159	4.3
	656	3	0.159	2.8
	639	4	0.107	7.6
	646	5	0.228	3.5
	648	6	0.137	2.2
Ayrshire	U-909	6	0.094	5.2
	919	7	0.115	4.0
	911	12	0.106	5.8
Goat			0.177	7.1

as far as possible the effect of any variation in the feed of the herd. The boron content of the feed was between 3 and 5 p.p.m.

A second experiment was carried out to determine whether or not boric acid fed to the cow would appear in the milk. The results from the runs on 4 Holstein cows are shown in table 4. The boric acid was fed in a capsule immediately after the evening milking. Milk samples were obtained

TABLE 4

The increase of the boron in milk by feeding H_3BO_3

A single dose of 10 grams H_3BO_3 (=1.75 grams boron) fed in capsule to nos. 33 and 63. Cows 10 and 11 were fed 13.5 grams H_3BO_3 (=2.35 grams boron).

COW NUMBER	NUMBER OF DAYS AFTER BORON FEEDING	MILKING PERIODS AFTER BORON FEEDING	BORON PER LITER	LITERS MILK PRODUCED	TOTAL BORON EXCRETED	PER CENT OF FED BORON SECRETED IN 24 HOURS
			mgm.		*mgm.*	*per cent*
33	0	0	0.150	0.60	0.09	
	1	1st	1.05	0.55	0.578	0.06
		2nd	1.00	0.50	0.500	
	2	4th	0.393	0.40	0.160	
	3	6th	0.190	0.40	0.076	
63	0	0	0.185	7.90	1.46	
	1	1st	1.110	8.20	9.10	0.90
		2nd	0.995	8.00	8.00	
	2	4th	0.410	7.60	3.12	
	3	6th	0.200	7.85	1.57	
10	0	0	0.090	4.25	0.38	
	1	1st	0.185	4.14	0.77	0.30
		2nd	1.320	4.55	6.01	
	2	4th	0.646	4.32	2.79	
	3	6th	0.198	4.60	0.92	
11	0	0	0.115	15.00	1.72	
	1	1st	1.56	15.50	24.18	1.6
		2nd	1.12	13.65	15.29	
	2	4th	0.370	15.80	5.85	
	3	6th	0.127	14.80	1.92	

at the next morning and afternoon milkings and the afternoon milkings thereafter until the boron concentration had returned to normal.

The boron content of eggs. A group of 20 hens which had been on a ration containing 1.30 mgm. of boron per kilo were placed on the same ration to which was added 0.5 per cent boric acid. All of the eggs from five of the birds were analyzed for boron. The results of these analyses are shown in table 5. The eggs produced during the first 24 hours after placing the hens on the high boron ration showed some increase in boron

content, but the large increases did not occur until the second day. Egg production in the entire flock ceased after 6 days. At this time the hens were returned to the previous normal ration, and 14 days later they began to lay again. Eggs obtained after the resumption of laying were analyzed and their boron content found to be in the normal range. The same hens were then placed on the basal ration plus 1 per cent of boric acid. This time egg production did not cease and the birds were kept on this level of boron for 20 days at which time the amount of boric acid in the ration was

TABLE 5

The boron content of eggs

Hens on a basal ration containing 1.3 mgm. boron per kilo were transferred on the indicated dates to the same ration plus the various levels of boric acid.

DATE	NUMBER OF EGGS ANALYZED	AVERAGE BORON CONTENT OF		RATIO YOLK/WHITE
		Whites	Yolks	
		mgm. per kilo, fresh		
1/14	3	0.325	0.041	0.13
15	2	0.200	0.024	0.12
16	2	0.209	0.020	0.10
1/16: 0.5 per cent boric acid added to ration				
17	4	0.470	0.10	0.20
18	3	9.33	1.39	0.15
19	4	12.1	1.57	0.13
20	2	16.0	1.38	0.09
21	3	13.8	1.37	0.10
23	1	8.2	1.90	0.23
1/23: Hens changed to basal ration				
2/12	2	0.224	0.042	0.19
4/24: 1.0 per cent boric acid added to ration				
5/3	4	13.8	2.29	0.17
13	3	14.0	3.75	0.25
5/14: 2.0 per cent boric acid added to ration				
16	1	14.7	6.60	0.45
25	1	23.3	2.63	0.11

increased to 2 per cent. The boron content of eggs produced while the hens were on these levels of boron is shown in table 5.

The transfer of boron through the placenta and milk of rats. In this experiment the fetuses and young of pregnant and lactating rats were analyzed for boron. About 50 gram samples of young rat (live weight) were minced in a platinum basin, taken to dryness in the presence of 10 cc. of a 1 N NaOH solution, and analyzed for boron. The stomachs containing milk were removed and discarded so that the values reported are strictly on the basis of the boron content of rat tissue. The mother rats were of three groups: *a*, on mineralized cow's milk; *b*, on mineralized milk plus 50 micrograms of boron per day (as boric acid)—

this supplement was given in a single dose in the morning; *c*, on stock ration which contained 5.3 mgm. boron per kilo. (Assuming that the mother rat ate 10 grams of feed per day she would be ingesting 53 micrograms of boron.) The essential difference between *b* and *c* is that in *c* the mother rat is consuming boron throughout the day while in *b* the same total amount of boron is consumed within the space of a few hours.

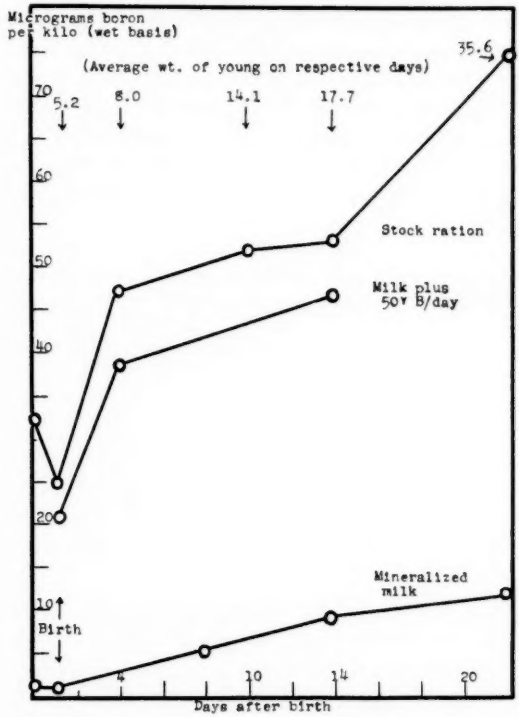

Fig. 2. The influence of the boron content of the diet on the transference of boron through the placenta and milk of rats. The points on the curve represent the boron concentration in fetus and young of lactating mothers on the indicated rations.

The results of this experiment are shown in figure 2. The fetuses analyzed were removed from the pregnant rat as close to the time of delivery as could be judged.

The rate of excretion of boron fed as boric acid to rats. The object of this experiment was to determine the length of time required for the complete elimination of 50 micrograms of boron (as boric acid) fed to rats on a basal ration of mineralized milk. At the time of analysis the rats weighed between 180 and 200 grams. The rats were starved for five hours and then

given 5 cc. of milk containing the 50 micrograms of boron. This was consumed in a few minutes. After varying periods of time the rats were killed, the entire GI tract removed, and the carcass ground in a carefully cleaned and washed meat grinder. This hash was placed in three platinum dishes, weighed, taken to dryness with alkali, ashed and analyzed for boron in the usual way. Some trouble was experienced in these analyses due to the formation of a sludge of insoluble calcium phosphate on taking the ash up in phosphoric acid. However, by bubbling a current of gaseous methyl alcohol through the sample during the distillation it is kept stirred up sufficiently. The results of this experiment are shown in table 6.

Boron in the nutrition of the rat. A number of litters of rats at the age of 2 weeks were placed with their mothers on screens. The mother rats were kept on mineralized milk to reduce the store of boron in the young. After weaning the young were started on a basal ration of whole cow's

TABLE 6

The rate of elimination of boron fed to adult rats on a basal diet of mineralized milk
Each figure represents the average of 4 to 8 analyses

TIME AFTER FEEDING 50 MICROGRAMS BORON (BORIC ACID)	BORON PER KILO	BORON PER RAT	PER CENT OF ADDED BORON STILL PRESENT IN RAT	BORON IN GI TRACT
	micrograms	*micrograms*		*micrograms*
0	11.4	2.5		
1 hour	205	40.9	82.0	6.25
2 hours	146	25.7	51.4	3.80
6 hours	84.1	18.8	33.6	1.55
28 hours	45.1	8.3	11.6	0.90
3 days	17.3	3.4	1.8	0.72

milk mineralized with iron, copper and manganese, with various levels of boron. The milk was received in a soft glass bottle. Added boron had no effect on the growth rate of these rats as is shown in table 7. The rats were carried through to reproduction and the reproduction efficiency of the females is shown in table 8. The 3 females receiving 1 mgm. of boron per day did not become pregnant although they were caged with a male of known potency for 6 weeks. A month after being taken off of the high boron supplement and kept on the basal, two of these females gave birth to normal young and reared them successfully.

There is sufficient boron in cow's milk to furnish a growing rat between 5 and 10 micrograms daily. (No boron could be detected in 30 cc. of the mineral supplement used, 0.5 cc. being the daily dose.)

Since milk is not as low in boron as could be wished for this type of work, an attempt was made to produce a dry ration low in boron. Acid precipitated casein was chosen as the source of protein, since Bertrand and Agulhon have shown (3) that the boron in milk remains almost entirely in

the supernatant whey. Analysis showed this casein to contain 0.15 microgram of boron per gram. Commercial sucrose contains about 0.16 microgram of boron per gram, which could not be diminished through several recrystallizations from alcohol. This may be explainable on the basis of formation of the so-called spiran compounds (Levy and Doisy, 13). A liver extract powder (obtained from Wilson and Company) was found to be free of boron within the limits of the method. The salt mixture used was the same as described by Todd, Elvehjem and Hart (14).

TABLE 7

Growth of rats on a basal diet of mineralized milk supplemented with various amounts of boron (boric acid)

DAILY SUPPLEMENT OF BORON	MALES		FEMALES	
	Number of rats	Grams/day (6 weeks average)	Number of rats	Grams/day (6 weeks average)
Control	8	3.25	7	2.73
20 micrograms	5	3.30	4	2.65
50 micrograms	6	3.23	4	2.53
100 micrograms	1	3.52	3	2.81
1000 micrograms	2	3.38	3	2.36

TABLE 8

The reproduction efficiency of female rats on a mineralized milk diet supplemented with various amounts of boron

DAILY SUPPLEMENT OF BORON	NUMBER OF FEMALES	NUMBER OF FERTILE FEMALES	NUMBER OF LITTERS PRODUCED	AVERAGE NUMBER OF PUPS/ LITTER	AVERAGE WEIGHT OF PUPS	PER CENT OF PUPS BORN RAISED TO WEANING
					grams	
Control	6	5	6	6.3	5.3	48
20 micrograms	3	3	6	6.8	5.4	64.5
50 micrograms	3	2	3	8.0	5.3	100
100 micrograms	3	3	4	7.2	5.5	54
1000 micrograms	3	0				

The boron content of this salt mixture was found to be 270 micrograms per kilo. The complete ration as used is as follows:

Casein	18
Sucrose	70
Salts	4
Liver extract	3
Corn oil	5
Vitamin B_1	1 microgram per gram ration
Haliver oil	2 drops weekly

The complete ration analyzed at 155 micrograms of boron per kilo. The casein, sucrose, salts and liver extract can account for about 130 micrograms of this. The remaining 25 micrograms must come from contamination during preparation and from the corn oil.

The growth of young rats on this ration is shown in table 9. Assuming a 5 gram daily food intake, the rats will be receiving 0.8 microgram of boron per day through their ration. The added boron rats, which were receiving 100 micrograms of boron per 75 cc. of drinking water, showed no better growth rate than the low boron rats. In studying reproduction of rats on this ration it soon became evident that an additional source of vitamin E was necessary so 1 per cent of wheat germ oil was added to the ration. These studies are now being carried forward and will not be reported at this time.

TABLE 9

Growth of rats on a boron low dry ration with and without added boron

	MALES		FEMALES	
	Number of rats	Grams/day (6 weeks average)	Number of rats	Grams/day (6 weeks average)
Basal	4	3.11	7	2.49
Plus 100 gamma boron per 75 cc. of drinking water	6	3.34	7	2.47

Discussion. The values which we have obtained for the normal boron content of milk, egg whites and egg yolks are in fairly close agreement with those reported by Bertrand and Agulhon (3). This is rather surprising since we have also shown that the boron content of these materials can be changed easily by increasing the boron in the feed. The explanation of the similarity in our values probably lies in a more or less constant boron content of plant materials used for feed purposes. Bertrand and Agulhon report that cow's milk contains two and one-half times more boron than human milk. It is interesting to speculate on how much of this difference is due to the types of food consumed, since all plant materials are considerably higher in boron than the animal products which make up a large part of the human diet.

It is difficult to reconcile our findings of a much higher boron concentration in whites than in yolks with Drea's (7) spectrographic detection of this element in yolk but not in white. Perhaps the fact that Drea added no basic material to his samples before ashing plays a part in his findings.

Kahlenberg (15) has shown that boric acid can diffuse with extreme rapidity through living animal membranes. The increase of the boron in the milk of cows fed boric acid is in agreement with this observation. Our

data on the increased boron content of milk due to the feeding of boric acid indicate that the excretion of boric acid from the blood into the milk is directly proportional to the secretory activity of the mammary gland. That is, the amount of boron per unit volume of milk secreted is constant whether the cow is milking heavily or is practically dry.

Since boric acid is so readily diffusible it is difficult to understand why there exists such a marked difference between the boron levels in the egg white and the egg yolk. No diffusion of the boron occurs even when the eggs have been allowed to stand at room temperature for 20 days before analysis. The most obvious explanation is that the boric acid is bound up in some non-diffusible compound in the egg white. From a teleological point of view it may be argued that the boron, being toxic, is placed in the white, rather than the yolk, for the protection of the embryo. If this were so, a decrease in the yolk to white ratio ought to occur when high boron rations are fed. Our experiments indicate that there is no significant change in this ratio.

In agreement with the results obtained from the experiments on cow's milk, it has been found that the boron content of rat's milk can be greatly increased by feeding boron to the lactating rat. It has also been observed that the amount of boron transferred through the placenta depends on the amount of boron in the ration. There is a greater ease of transference of boron into the milk than through the placenta. This is shown by the sharp increase in the boron concentration of the young a day or so after birth. The second sharp increase, beginning on the fourteenth day after birth, is due to the fact that the young now have their eyes open and partake of their mother's stock ration. This increase is not noticed in the case of the young of the rats on the mineralized milk diet, thus indicating that, in this case, the boron concentration in rat's milk is approximately in the same range as in cow's milk.

Our work on the rate of elimination of fed boron confirms the previous observations in the literature on the extreme rapidity of this process. Two hundred gram rats on mineralized milk contain only 2.5 micrograms of boron in their entire body. This represents a boron concentration of 11.4 micrograms per kilo of live weight. Since weanling rats whose mothers have been on mineralized milk show a boron concentration of about 12.0 micrograms per kilo live weight it certainly seems that there is no increase in boron concentration with age.

Weanling rats placed on a mineralized milk ration show no better growth than litter mates receiving amounts of added boron ranging from 20 to 1000 micrograms per day. Some evidence was obtained, however, that the ability of these rats to successfully raise a litter of young was increased when the smaller amounts of boron were fed. Apparently the 1 mgm. of boron per day level had a sufficiently toxic effect to prevent impregnation.

About 5 to 10 micrograms of boron per day is taken in by a growing rat on a mineralized milk basal ration. A dry ration has been prepared which permits growth equivalent to that attained on the milk ration, but which allows only 0.8 microgram of boron per rat per day. Since supplementing this ration with boron did not better the growth rate, it can be concluded that if boron is needed by the growing rat, then 0.8 microgram per day satisfies this requirement.

SUMMARY

1. A colorimetric method for the determination of boron in biological materials has been described. This method is subject to a 10 per cent error between 0.5 and 5 micrograms.

2. The milk from 22 cows has been analyzed for boron. There is no significant difference between breeds, nor for different periods of the lactation cycle.

3. It is possible to increase the boron concentration of the milk tenfold in the first 24 hours after feeding 13 grams of boric acid.

4. Normal egg whites contain from 8 to 10 times more boron than egg yolks. This ratio is not altered significantly when the hens are placed on a high boron ration, although the absolute amounts increase greatly.

5. Boron, fed as boric acid, can pass through the placenta and can pass into the milk of rats. The extent of passage into the milk is somewhat greater than its passage through the placenta.

6. Boric acid fed to rats is eliminated with extreme rapidity.

7. The addition of boron to milk does not produce better growth in young rats. There is some evidence to show that young are better able to survive the nursing period when their mothers are receiving supplements of boron. This point needs further study.

8. The addition of boron to a dry ration which allows only 0.8 microgram of boron per rat per day does not result in better growth. It can be concluded that if boron is needed by the growing rat then this amount of boron per day satisfies that requirement.

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GONADOTROPIC HORMONE IN THE NON-PREGNANT MARE

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Earlier studies showed that the concentration of gonadotropic hormone in the blood of non-pregnant mares was too low to determine without using extraction methods (Cole and Hart, 1930). In 1936 Dr. I. P. Earle, in conjunction with the senior author, attempted to study the gonadotropic hormone level in the blood and urine of non-pregnant mares and cows using the methods of extraction available at the time. By none of these methods were we able to demonstrate the existence of gonadotropic hormone in non-pregnant animals.

In 1937 Cartland and Nelson devised a greatly improved method for concentrating gonadotropic hormone in pregnant mare's blood. We have concentrated, in batches of one to four liters, between one and two hundred liters of blood serum from pregnant mares by their method and usually obtain 50 to 75 per cent recovery. In a few instances we found recoveries as low as 25 per cent. Consequently we have used a slight modification of their method in the present studies by which positive responses are fairly regularly obtained. By this method sufficient purification is obtained to make possible the injection of the equivalent of one-half liter of blood serum into a single rat.

The question arises as to what hormones are extracted. A popular viewpoint is that expressed by Fevold and associates, who maintain that the gonadal response is dependent upon the dual action of two hormones, FSH and LH. Our studies indicate that either both are regularly present in approximately the same relative amounts or that a single hormone, differing from FSH and LH, is present. We have also considered the question of the completeness of extraction.

METHODS. Both Thoroughbred and draft mares were used, most of them being two or three years old. They were tested daily for estrus with a stallion. The length of the cycle was determined in most instances and found to be 18 to 21 days except for mare 35 in which estrus had not recurred at 37 days after the beginning of the preceding heat. Also mares 39 and 40 did not come into heat over a 70-day observation period. The average length of estrus was 4.2 days with extremes of 2 and 8 days.

Between 2 and 3 liters of blood were obtained every 4 to 7 days through-

out the cycle. We wished to keep the amount drawn at a minimum in order to reduce the effect on the hormone level of subsequently drawn blood. The blood was defibrinated by whipping and the serum separated from the cells by centrifugation. From this point a modification of the Cartland and Nelson (1937) method was employed.

The pH of the serum was adjusted to 9.0 and an amount of acetone equal to 90 per cent of the serum volume was added slowly with vigorous stirring. After standing 18 hours at room temperature the precipitate was separated from the supernatant liquid by means of flannel filter bags in a hand press and washed with a volume of 50 per cent acetone (sp. gr. 0.937) equal to the original serum. The precipitate was discarded. The supernatant liquid was then carefully brought to 50 per cent acetone, adjusted to pH 6 and allowed to stand over night at 34°F. The following morning the precipitate which had formed was separated in a supercentrifuge and discarded. The filtrate was brought to 70 per cent acetone (sp. gr. 0.897). After standing for 2 to 4 days the resulting precipitate was collected by centrifugation and dissolved in an amount of saline representing one-fiftieth the original volume of serum. The approximate concentration of gonadotropic hormone was determined by injecting amounts of the extract equivalent to 50, 100 and 200 cc. serum into 25-day old female rats. All rats were sacrificed 120 hours after initial injection. This was followed by further tests to determine the potency of the serum more accurately though the concentration was too low to make feasible the assay of the serum by previously published methods. Some extracts were also injected into immature male rats to determine their effect upon the seminal vesicles. To check the completeness of extraction many of the sera were injected both untreated and after extraction into both male and female rats.

RESULTS. *Effects of extracts on immature female rats. Serum from mares with normal cycles.* The amount of serum equivalent of the extracts necessary to produce an ovarian or vaginal response is shown in table 1. Only rarely was a response obtained with less than 100 cc. of serum equivalent of extract. Twenty-four out of 35 gave a positive vaginal response but only eighteen a positive ovarian response at the 200 cc. level. In ten instances a positive ovarian response was not obtained at any level tested, whereas only four extracts failed to give a positive vaginal response at the highest level. In other words, a number of rats injected with the extracts had estrous smears but no ovarian change detectable with a dissecting microscope. This is also a characteristic response of minimal doses of pregnant mare serum and, because of the good agreement in general between ovarian and vaginal responses, it is fairly certain that the extracts did not contain appreciable amounts of estrogen. It is clear from the data that no marked changes occur in the concentration of gonadotropic hormone dependent upon the estrous cycle in mares having normal cycles. There is an indication that the concentration of hormone is greater during metoestrum than during estrus though many more tests would be necessary to obtain statistically significant figures. As stated earlier, the average length of estrus was 4.2 days.

TABLE 1

The ovarian and vaginal response of immature female rats to extracts of mare serum collected at various periods of the estrous cycle

A positive ovarian response refers to the presence of ripe follicles or corpora. A positive vaginal response refers to an estrous smear. The number of rats used at a given level is indicated by the number of plus and minus signs.

MARE NUMBER	DATE BLED	DAY OF CYCLE*	OVARIAN RESPONSE TO THE FOLLOWING DOSES OF CC. SERUM EQUIVALENT:						VAGINAL RESPONSE TO THE FOLLOWING DOSES OF CC. SERUM EQUIVALENT:					
			30	50	100	200	300	400	30	50	100	200	300	400
31	5/ 8/39	1		−	−	−	−	−		−	−	−	−	+
32	2/17/39	1		−	+		+	+		−	+		+	+
33	4/ 7/39	1		−−	−−	+−−				−−	−+	−++		
31	3/16/39	2		−	−−	−−	+			−	−−	−+	+	
34‡	4/ 7/39	2		−−	−−	−−	−			−−	−−	++	+	
35	4/19/39	2		−	−	−	+	+		−	−	−	+	+
36	4/26/39	4	−	−	−	−	++			−−	+	+	++	
31	4/26/39	6		−	−−	−−				−	−	+−	+	
33	4/12/39	6		−−−	+	+				−+−	−−	+		
				−−−	−−−					−−−	++			
31	3/21/39	7		−−	+++	+				−−	++	+		
				−−	−					−−	++			
34‡	4/12/39	7		−−	+	+				−−	+	−		
					−−	−−−					−−	+++		
35	4/26/39	9		−	−	+−		+		−	−	+−		+
36	5/ 2/39	10		−	−	−	+−			−	−	+	+−	
33	4/17/39	11		−	+−−	+++				−	−+−	+++		
34‡	4/17/39	12		−	−	−	−			−	+	+	+	
31	5/ 2/39	12		−	−	−−	−			−	−	−−	−	
31	3/27/39	13		−	−−−	+++				−	−−−	+++		
						+						+		
37	2/17/39	14†	+		+	+		+	+		+	+		+
38	2/17/39	15†		−	+		+			−	+		+	
35	5/ 2/39	15		−	−	−	−	−		−	−	−	−	+
36	5/ 8/39	16		−	−	−	−	+		−	−	−	−	+
31	4/ 1/39	18	−−−	+++	+	+			−−−	+++	+	+		
33	4/24/39	18		−	−	−	−	−		−	−	−	−	−
34‡	4/24/39	19		−	−−	−−		−		−	−−	−−		−
35	5/ 8/39	21		−	−	−	−	−		−	−	−	−	−
35	5/12/39	24		−	−−	+−−				−	−−	+++		
					−−						−−			
35	5/19/39	31	+	++	++	+		++	+	++	++	+		++
35	3/24/39	Unknown§		−−+	++	+				−++	++	+		
				+++						+++				
35	4/ 3/39	Unknown§		−	−	+−		−		−	−	+−		+
35	4/ 7/39	Unknown§		−	−	−−−				−	−	−−−		
39	3/29/39	No cycle¶		−	−	−		+		−	−	−		+
39	4/ 3/39	No cycle¶	−	+−	++	+			−	++	++	+		
39	4/24/39	No cycle¶		−	−	−	−	−−		−	−	−	−	+−
40	3/29/39	No cycle¶		−	−	−+	+	+		−	−	−+	+	+
40	4/ 3/39	No cycle¶	+++	+++	+	+			+++	+++	+	+		

* The first day of heat is designated as the first day of the cycle.

† The time of the preceding estrus was unknown and the time was reckoned on the basis of a 21 day cycle from the heat period following the bleeding.

‡ This mare was bred on 4/7/39 and may have become pregnant.

§ Observations for estrus began on this mare on 3/16/39. Her first estrus was on 4/18/39.

¶ This three year old mare was observed over a 70 day period but showed no evidence of estrus.

Mares with abnormal cycles. The data for some of these mares are given in table 1 but for convenience we have summarized the data for mare 35 in table 2.

The interesting point here is the relatively great variation in hormonal concentration as compared with mares having normal cycles. For example, 400 cc. of serum equivalent drawn from mare 35 on May 19 produced ovaries in two rats weighing 90 and 66 mgm. respectively, whereas 400 cc. serum equivalent drawn on May 8 was entirely negative. Ovarian weights of this magnitude are produced by 16 rat units of pregnant mare serum.

Variations of a similar magnitude occurred in two other mares (39 and 40) which did not come into estrus during the observation period (table 1).

Several years ago we had 3 mares whose blood serum gave a positive response at the 30 cc. level. All of these mares had been bred and a long

TABLE 2

Date observations for estrus were begun, March 16, 1939; date of first observed estrus, April 18, 1939; date of subsequent estrus, no estrus for 37 days after 1st heat at which time observations were discontinued.

DATE OF BLEEDING	DAY OF CYCLE	AMOUNT OF SERUM TO PRODUCE AN OVARIAN RESPONSE
		cc.
3/24	Unknown	50
4/ 3	Unknown	100
4/ 7	Unknown	>200
4/19	2	300
4/26	9	200
5/ 2	15	400
5/ 8	21	>400
5/12	24	200
5/19	31	<30

interval with no estrous periods intervening had elapsed before samples were taken. The protocols are shown in table 3.

In the first of the three mares the concentration of hormone remained constant over the observation period whereas in the last two the relatively high concentration was transient. These cases give further evidence that high concentrations of hormone are associated with irregularity or cessation of cyclical activity.

Completeness of extraction. In order to determine the loss of hormone due to extraction ten untreated sera collected at various phases of the cycle were injected into immature female rats in total doses of 60 and 30 cc. over a 3-day period. The results were compared with those obtained with the extracts. Sixty cubic centimeters represent a maximal amount which can be introduced in a 25-day old rat without injurious effects. Although this was below the serum equivalent of extract necessary to produce a re-

sponse in most instances still it gives some indication of the amount of hormone lost during extraction. Though no positive ovarian responses were obtained estrous smears were found in rats at both levels injected with serum from mare 36, April 26. Two sera gave negative responses at the 60 cc. level whereas 50 cc. of serum equivalent of extract gave positive ovarian responses. Rather than assuming that the extraction removed an inhibitory substance we believe that the extract was more effective because of the fact that the entire dose was given in a single injection while it was neces-

TABLE 3

DAYS AFTER BREEDING	SERUM	RESPONSE	NUMBER OF RATS
Lottie—21 year old mare. Bred May 25, 1935			
	cc.		
43	30	Positive	2
46	30	Positive	6
	10	Negative	6
74	30	Positive	1
	5	Negative	1
74	sacrificed and found no evidence of fetus or implantation site. One large corpus luteum was present		
Windy—old mare—age unknown. Bred August 25, 1935			
41	30	Positive	2
46	30	Positive	2
	10	Negative	1
60	30	Negative	1
60	sacrificed and found to be non-pregnant and a condition of pyometra was encountered. The uterus contained between one and two quarts of fluid. One large corpus luteum was present		
Hamel—old mare—age unknown. Bred July 26, 1935			
40	30	Negative	2
45	30	Positive	1
	10	Negative	1
53	30	Negative	1
	10	Negative	1

sary to make multiple injections of the serum. We have previously shown that at the minimum level a single injection of pregnant mare serum is more effective than multiple injections.

Seven untreated sera were injected into immature male rats using the same dosage as was used on the females. The seminal vesicles of all rats remained infantile.

The assumption is frequently made that there are two gonadotropic hormones, FSH and LH. FSH is considered to be more effective in the

female whereas LH is presumably more effective in the male. The two hormones presumably augment each other in either sex. If our method of extraction resulted in a loss of one or the other, one would expect the untreated serum to react at lower levels than the extract. We found no evidence of this though we were not able to inject as much untreated serum as serum equivalent of extract in all instances. We do not wish to infer that the extraction involves no loss of hormone. With the Cartland and Nelson method we usually obtain 50 to 75 per cent recovery. Our modification has proved more effective in our hands, but some of the variations in hormone content reported may be due to loss during extraction.

On the biological nature of the hormone in non-pregnant mare serum. We have been unable to obtain any evidence that the hormone in the non-pregnant differs from that in the pregnant mare. Its response in the immature female rat appears identical with that of pregnant mare serum: At minimal levels a positive vaginal response is frequently obtained even though ripe follicles or corpora are lacking. When two to four times the amount necessary to produce a positive vaginal response is given corpora lutea are almost invariably found, irrespective of the phase of the cycle at which the blood was drawn. The ovarian weight is still not appreciably affected by this dosage and this is also characteristic of pregnant mare serum. Only in a few cases were we able to inject still larger amounts. For instance, extract A78 (see table 1 for amounts to produce minimal responses) produced ovaries weighing 76 mgm. when 200 cc. serum equivalent were injected; extract A80, 36 mgm. ovaries with 200 cc.; A88, 32 mgm. with 200 cc.; A105, 90 mgm. with 400 cc. given subcutaneously and 60 mgm. with 400 cc. intraperitoneally. Both corpora lutea and ripe follicles were found in the ovaries of rats receiving these large doses. The single instance in which the extract was given intraperitoneally is significant because of Fevold's recent report that pituitary FSH is ineffective by this route (Fevold, 1939a). If the gonadotropic principle of mare serum consisted of a combination of FSH and LH having the properties described by Fevold one would not expect a response by this route of administration.

Fevold (1939b) believes that some idea of the relative amounts of FSH and LH present can be obtained by comparing the male and female response. Consequently the following extracts have been injected not only into females but also into males: A75, A76, A77, A78, A79, A80, A81, A83, A87, A88, A90 and A91. Seminal vesicle responses were obtained with approximately twice the amount necessary to produce a minimal response in the female. This again is comparable to pregnant mare serum (Cole, 1936). It was not possible to obtain a positive response with all extracts because of the low titre of the hormone.

Discussion. The data submitted indicate that the blood serum of

non-pregnant mares contains 2 to 30 rat units per liter as compared to 25,000 to 100,000 for the pregnant mare at the 70th day. Variations of hormone concentration dependent upon the cycle are apparently slight though there is some indication of an increased concentration during the first week of metoestrum. We have been unable to detect changes in the qualitative response to injected serum dependent upon the phase of the cycle at which the blood was drawn. The response of the serum can best be described as being identical with that of pregnant mare serum.

We interpret this to indicate that the non-pregnant mare does not secrete FSH at one phase of the cycle and LH at another as postulated by Hisaw (1935) and Fevold, Hisaw and Greep (1936) for certain species but that the mare secretes only one gonadotropic hormone.

We believe this represents the first study of gonadotropic hormone in the blood of normal non-pregnant animals using a method by which positive responses are obtained. There are a number of negative reports in which untreated serum was used. To our knowledge no one has given satisfactory evidence that the gonadotropic principle in the blood serum of any species can be separated into FSH and LH fractions. We do not wish to infer that unconfirmed reports are lacking. Fevold (1939) has cited those referring to the separation of pregnant mare serum including two reports from our own laboratory but failed to cite two of our more recent papers in which we ascribe the augmenting action of the second fraction in mare serum to inert protein rather than to a gonadotropic hormone (Saunders and Cole, 1936, and Saunders and Cole, 1938). Fevold and Fiske (1939) have recently submitted evidence which they interpret as indicating that estrogen will increase the LH present in the blood serum of rats. Extracts of serum obtained from rats treated with estrogen gave a positive seminal vesicle response but a negative ovarian response. We injected both males and females with untreated serum as well as extracts of the serum in the present study but found no evidence of this nature.

We had anticipated finding either variations in the amount of FSH and LH or greater variations of a single gonadotropic substance at different periods of the cycle. Perhaps the results indicate that none of the theories concerning the control of the estrous cycle is entirely adequate.

CONCLUSIONS

1. Gonadotropic hormone is present in the blood serum of non-pregnant mares at all phases of the estrous cycle. There is some indication that the greatest concentration is reached during metoestrum.

2. The hormone could not be distinguished biologically from that found in the pregnant mare.

3. The highest concentrations of hormone were found in mares with pro-

longed cycles or in mares which failed to come into estrus during the observation period.

4. No evidence was found that mare serum contained two gonadotropic hormones, FSH and LH.

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THE EFFECT OF EXTRACTS OF THE ADRENAL GLANDS ON THE RESPIRATION OF ISOLATED BRAIN AND LIVER SLICES[1]

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It is well known that adrenalectomized rats have a lower basal metabolic rate than normal rats. Following treatment with extracts of the adrenal cortex the normal heat production is restored (for literature see 1). More recently Carr and Beck (2) have published results showing that the B.M.R. after adrenalectomy decreases progressively until death. The rate after adrenalectomy reached a level averaging 25 per cent below that before adrenalectomy. The average R.Q. was lower after the operation than before. The injection of cortin has little or no effect on the B.M.R. of normal dogs (3) or of normal human beings (1, 4). However, the oxygen consumption of human beings during quiet standing or while exercising on a treadmill is less during cortin injections than before or after the treatment (1). The capacity for anaerobic work is not affected (4).

Adrenal insufficiency results in marked neural disturbances as reflected in neuro-muscular performance and in the development of conditioned reflexes (5, 6). It was thought of interest to study the metabolism of isolated brain slices under the influence of added adrenal extract. The results indicate that the extract significantly depresses the oxygen consumption of brain and liver slices. The depressing effect appears to be due to a combination of a nonspecific factor present in adrenal extracts and in brain extracts, and a factor specific for the adrenal cortex. The R.Q. of brain slices is not affected by the extracts.

METHODS. Thin slices of cerebral and liver tissue of rats were prepared in the customary manner initiated by Warburg (7). The respiration was measured in differential microvolumeters (8) at a temperature of 37.5°C. The animals were killed by cutting off their heads and the tissues rapidly prepared while lying on a cold plate. The first respiratory recording was made within thirty minutes after the death of the animal. Albino rats of Wistar stock were used in all experiments. Males were used exclusively ranging from 150 to 250 grams. In all experiments the animals were starved for twelve hours preceding the test. Mammalian Ringer buffered with 0.02 M phosphate buffer at pH 7.3 to which was

[1] Aided by a grant from the Rockefeller Foundation.

added 0.02 M dextrose was used. Two cubic centimeters of medium were used in experimental flasks. The medium was equilibrated with pure oxygen, and the respiration was measured with pure oxygen in the flasks. The respiratory CO_2 was absorbed by 10 per cent potassium hydroxide. The R.Q. was determined by the method described by Dickens and Simer (9). The tissue slices were dried at 110°C. for twelve hours to determine the dry weight. Oxygen consumption is expressed as Q_{O_2}. The initial and final pH of the medium was determined by indicator.

The tissue extracts were made in such a manner as to exclude the sodium factor in all extracts (10), with one exception. The exception was a fairly crude extract containing both adrenal cortex factors. Four adrenal extracts were used. One extract, as mentioned above, was a crude extract of the whole gland of the pig. A second extract was a further purification of this crude extract carried out in such a way as to exclude the sodium factor. A third extract was a highly purified whole gland extract of pig adrenal, while the fourth extract used was a highly purified

TABLE 1

The oxygen consumption of brain samples taken from the same brain

ANIMAL NUMBER	SAMPLE 1	SAMPLE 2	SAMPLE 3	SAMPLE 4
	c.mm. per mgm. per hour	*c.mm. per mgm. per hour*	*c.mm. per mgm. per hour*	*c.mm. per mgm. per hour*
3	7.8	7.3	7.5	8.1
6	8.1	8.7	7.8	7.1
15	10.1	9.4	10.3	9.7
37	6.2	6.67	5.8	6.0

extract of beef adrenal cortex. The solid content of the purified extracts ranged between 0.7 and 1.1 mgm. per cc. The three purified extracts gave negative tests for adrenaline (less than 1 part in 1 million). The extract of beef brain was prepared by the same method as the three purified concentrates of adrenal gland. It contained only 0.3 mgm. of solid per cc., and showed negative tests for adrenaline and sodium factor. Four cubic centimeters a day did not maintain adrenalectomized cats. All extracts were assayed for sodium factor (11) and for vital hormone (12).

OBSERVATIONS. *Adrenal extracts.* The oxygen consumption of the brain slices varied considerably from one rat brain to another. The average Q_{O_2} of brain slices from a group of 60 normal rats with no added extract was 8.45 with a maximal value of 11.2 and a minimal value of 6.5. The standard error of the mean was $\pm$ 0.186. Such large variation made it necessary to select control slices and experimental slices from the same cerebral lobes. Comparable slices selected for thinness and for uniformity taken from the same brain showed very similar respiratory values, as four representative experiments show in table 1. Samples taken from

the same brain showed an average variation of 6 per cent with an infrequent maximal variation of 10 per cent. Each flask contained about 100 mgm. of tissue wet weight so usually more than one slice was used in each experimental flask.

In table 2 are presented the data from experiments in which adrenal extracts of differing potencies of vital hormone were added to the tissue

TABLE 2

The oxygen consumption and R.Q. of rat brain slices under the influence of adrenal extracts and of corticosterone

VITAL ACTIVITY OF EXTRACT	SOLID CONTENT OF EXTRACT	Q_{O_2}		R.Q.	CORTICOSTERONE	Q_{O_2}
c.u. per cc. of medium*	*mgm. per cc. of medium*				*mgm. per cc. of medium*	
None (control)	None	7.5	9.1† 6.4	0.99	None (control)	9.1
0.4	0.07–0.5	7.7	8.6 6.2	0.95	None (2.5 per cent alcohol)	8.5
1.0	0.09	7.3	8.0 6.0	1.05	0.05	7.8
2.0	0.35–0.5	6.2	6.8 4.7	0.98	0.125	7.25
4.0	0.7 –1.1	4.4	5.0 3.5	0.98	0.25	5.7
8.0	1.1 –2.5	2.0	3.0 1.8	0.95	0.5	4.5

* Cat maintenance units.

† The maximal and minimal values for each group of data.

slices. The control samples and the experimental samples for each individual experiment were taken from the same brain. The first values for Q_{O^2} (column 3) are averages of all the tests. The second ones are maximal and minimal values under each set of conditions. Averaged values for the R.Q. are included. It is quite apparent that extracts showing a potency in vital hormone of 2 cat units per cc. of medium (2 cc. of medium in experimental flask) and above, depress the oxygen consumption but have little influence on the R.Q. In many experiments the effect was progres-

sive, a greater effect being manifested the second hour than the first. The data clearly indicate a threshold at 2 cat units per cc. medium. There is varying sensitivity from animal to animal since some few were not affected by extracts of 2 c.u. but the respiration was depressed by higher concentrations. The respiratory effect was manifested equally by pig and by beef adrenal extracts. As is shown in table 2 the solid content of the extracts varied considerably without any apparent variation in respiratory effect. For example, one extract (2 c.u./cc. extract) used in volume to give 0.4 c.u. per cc. of medium contained as much solid material as that of another extract (8 c.u./cc. extract) used in sufficient volume to give 2 c.u. per cc. of medium, yet the former has no respiratory effect while the latter has an appreciable effect. The only known factor that consistently correlated with the respiratory effect was the vital factor. Therefore the data

TABLE 3

The oxygen consumption of brain slices from adrenalectomized rats in presence of adrenal extracts

VITAL ACTIVITY OF EXTRACT	ANIMAL 1 Q_{O_2}	ANIMAL 3		ANIMAL 4	
		Q_{O_2}	R.Q.	Q_{O_2}	R.Q.
c.u. per cc. of medium					
Control	6.75	7.98	1.1	9.55	0.98
0.8		5.88	0.95	6.25	1.05
2.0	5.75	4.88	1.05	4.48	0.95
4.0	4.30	3.59	0.98	5.0	0.99
Days after operation	6	10		16	

have been grouped according to their cortin content. The final pH of the medium was never less than pH 7.0.

The same action was manifested on rat liver slices although the experiments have not been so extensive with this tissue.

Essentially the same respiratory effect was manifested on brain slices from 3 adrenalectomized rats as evidenced by the data in table 3. The degree of insufficiency was determined by the amount of weight lost by the operated animals as compared to unoperated litter-mates as controls. The operated animals were used 6 to 16 days after the operation at which time they showed from 18 to 25 per cent loss in weight. Adrenalectomy appears to have little effect on the oxygen consumption of the brain slices since the Q_{O_2} values for control samples fall within the range for normal brain tissue. It is realized however that three experimental animals do not offer very conclusive evidence. There is a suggestion that adrenalectomized tissue has a lower threshold than normal tissue. The R.Q. of experimental samples was about the same as that of control samples.

Brain extract. One cubic centimeter of brain extract depressed the

respiration of brain slices from normal rats on the average 15 per cent, which is approximately equal to the effect of an adrenal extract containing 2 c.u. of cortin per cc. medium. One-half cubic centimeter had no significant effect while 2 cc. had about the same respiratory effect as the 1 cc. The adrenal extract is much more effective since much smaller quantities give even larger respiratory effects.

Corticosterone. Corticosterone dissolved in 2.5 per cent ethyl alcohol made up to desired volume in hypertonic Ringer so as to give an isotonic medium was tested for respiratory effects. It was necessary to dissolve the corticosterone in alcohol but controls showed that this concentration of alcohol had very little respiratory effect. The data are given in table 2, showing that corticosterone has a marked depressing action on the respiration of brain slices. The degree of inhibition varies with the concentration of corticosterone. The respiratory inhibition increased with time, the effect being more marked during the second hour than during the first. It may be significant that the solid content of the adrenal extracts falls in the same range as the amount of corticosterone which gives appreciable respiratory depression.

Discussion. The values of oxygen consumption reported in this paper are lower than those usually reported for cerebral cortex slices (13). However it was impossible to be sure that the slices contained only grey matter. The mixture of grey and white matter may explain the low values, and may partially explain the large individual variation observed.

The respiratory depression resulting from adrenal extract is consistent with the observations of Francke (3) and Hitchcock et al. (1) that injections of cortin decrease the total metabolism under certain conditions.

That the vital hormone may be the functional agent and not other substances present is suggested by *a*, the variation of the respiratory effect with vital activity; *b*, the activity of corticosterone, which may have some vital activity (14), although tests in this laboratory indicate practically none; and *c*, the absence of any appreciable effect of variation in the source, purification, or solid content of the adrenal extract.

Against such suggestive evidence may be raised the following points: *a*, an extract of brain tissue having no vital activity has some respiratory effect; and *b*, the respiratory effect of corticosterone is much greater than its vital activity.

The amount of corticosterone is approximately equal to the solid content of the adrenal extracts. If all the solid material in the adrenal extracts were corticosterone or substances of that nature, that alone might explain the respiratory effect of the extracts. That seems improbable since corticosterone has sodium retention activity (14) and is insoluble in aqueous solution, while the adrenal extracts had no sodium factor and contained solid material that was easily soluble in water.

The removal of the brain from the body and its subsequent injury by slicing may raise the threshold considerably, so it is possible that injections of extract might have respiratory effects *in vivo*.

Mune (15) reported respiratory inhibition of liver, kidney, spleen and muscle tissue of rats by added adrenal cortex extracts. (It has not been possible to read the original paper.) Oral administration of adrenal cortex powder (0.3 gm./kgm.) for 10 to 30 days diminished Q_{O_2} of all the tissues studied. One subcutaneous injection of 10 per cent adrenal cortex extract (1.5 cc./kgm.) lowered the Q_{O_2} of the tissues.

SUMMARY

Extracts of the adrenal whole gland and cortex depress the oxygen consumption of brain and liver slices of normal and of three adrenalectomized rats. The degree of inhibition appears to be proportional to the vital activity of the extract above a certain threshold of 2 c.u. per cc. of medium. The respiratory effect did not vary significantly with any other known variable in the extract. The R.Q. of the brain slices was not affected by the extract. An extract of beef brain also showed depressing effects on the respiration but to a much lower degree. Crystalline corticosterone in concentrations of the same magnitude as the solid matter of the adrenal extracts also showed marked depressing action.

I want to acknowledge gratefully many invaluable suggestions from Prof. F. A. Hartman, and technical assistance from Mr. Dan Strelnick.

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THE EFFECT OF THE INGESTION OF GLYCINE, WITH AND WITHOUT UREA, UPON CREATINE-CREATININE EXCRETION IN THE RAT AND MAN[1]

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As the result of an extensive study in this laboratory (1) we have shown that the following substances after parenteral injection resulted in the following average per cent increases in the concentration of muscle creatine in the rat: 100 mgm. glycine, 32; 100 mgm. urea, none; 100 mgm. glycine + 100 mgm. urea, 52. Sarcosine, in similar doses, with and without urea, resulted in larger increases than did glycine. Upon the basis of these results we have suggested that urea condenses with either glycine or sarcosine in the body in the synthesis of creatine. We are reporting here the results of urine studies after the ingestion of glycine, with and without urea, in both the rat and man.

EXPERIMENTAL. Three groups of young adult white rats weighing from 150 to 200 grams were fed upon our stock diet after weaning (1). Each group (6 animals) was then fed in the metabolism cages upon this stock diet with 5 per cent urea in the diet of group 1, 5 per cent glycine in that of group 2 and 5 per cent urea and 5 per cent glycine in that of group 3. During the second period of observation the animals were fed the same stock diet, but the amounts of urea, glycine, and urea and glycine added to it were increased to 10 per cent each. Urine samples were collected in 2 day periods under toluene and analyzed for total creatinine by autoclaving the sample with HCl, followed, after neutralization, by the addition of alkaline picrate. Preformed creatinine was determined by adding alkaline picrate to the fresh urine sample. All color comparisons of creatinine were made in the Fisher electrophotometer. During the studies 1 rat from each group was sacrificed and creatine (total creatinine) in a sample of muscle tissue was determined by the method of Rose, Helmer and Chanutin (2).

Subjects P. P. and H. H. B. ingested 5 grams of glycine in water during the first period, and 5 grams of urea during the second period. H. H. B.

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ingested 5 grams of urea + 5 grams of glycine during the third period. No change in the usual diet was made. Urines were collected in 2 day periods and analyzed for total and preformed creatinine as stated above. In three of these studies the color of the Jaffe reaction with alkaline picrate

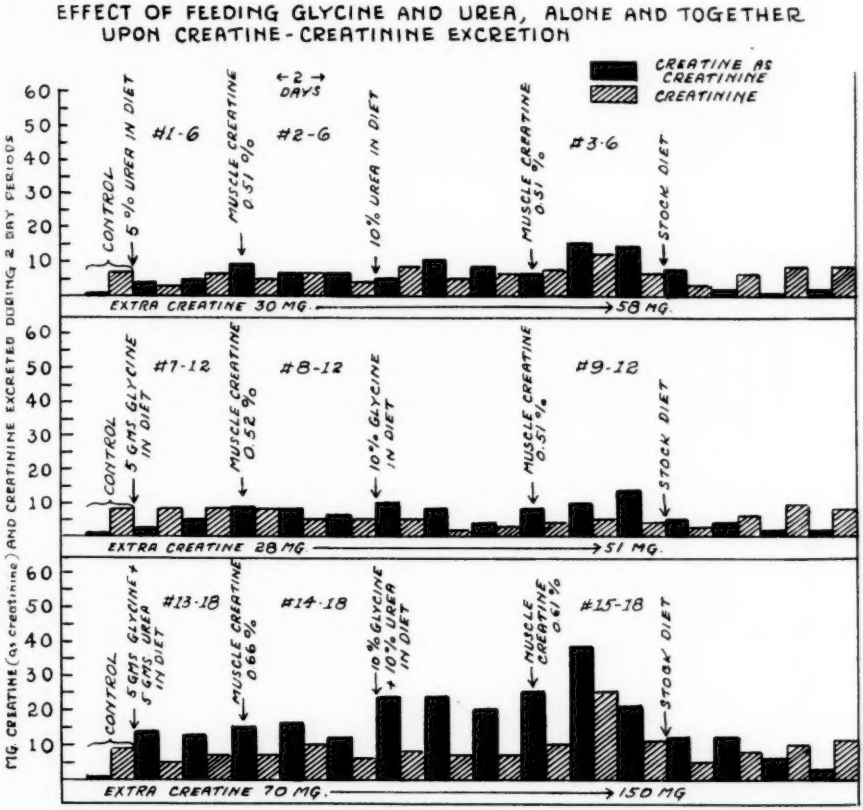


Fig. 1. Effect of feeding glycine and urea, alone and together, upon creatine-creatinine excretion.

was shown to be due to creatinine alone after incubating the sample with the M. and D. specific creatinine enzyme (3).

Results and discussion. The results of the animal experiments are given in figure 1. In group I the ingestion of the stock diet containing 5 per cent urea resulted in an increase in creatine excretion of 30 mgm., and the ingestion of the same diet with 10 per cent of urea caused almost twice as much creatine excretion, namely, 58 mgm. Similar results were also

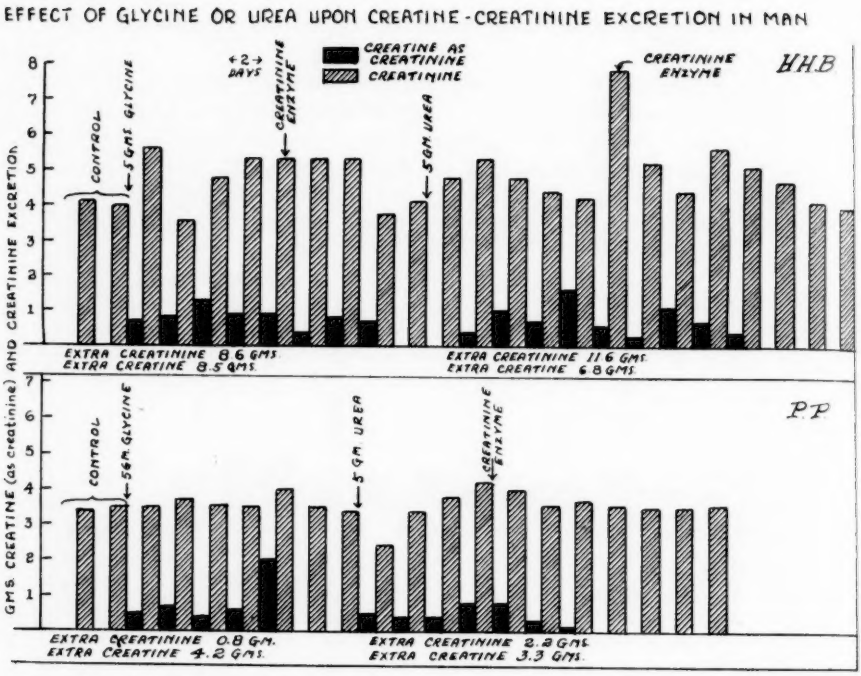


Fig. 2. Effect of glycine or urea upon creatine-creatinine excretion in man

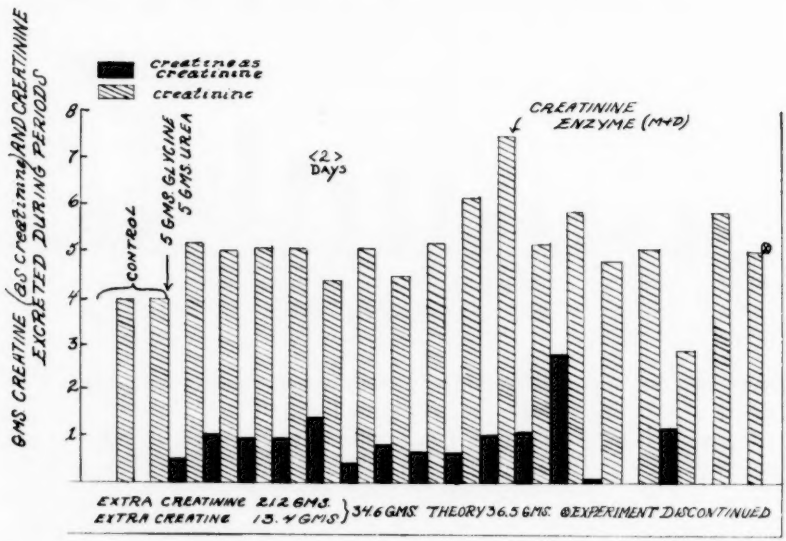


Fig. 3. Effect of glycine with urea upon creatine-creatinine excretion in man

obtained with these amounts of glycine. In group 2 ingestion of the diet with 5 grams of urea and 5 grams of glycine gave an increase of 70 mgm. in creatine excretion and the ingestion of the diet with 10 grams of urea and 10 grams of glycine resulted in a creatine excretion of 150 mgm. The usual increases in muscle creatine also occurred in these experiments. At the completion of the study the stock diet was again ingested, after which the creatinuria soon disappeared.

These results indicate that creatine production in the animal body, as determined by the extra creatine excretion, is directly proportional to the concentration of glycine or urea fed, separately and together, in the diet. Increases in creatinine excretion did not occur, except in a single 2 day period, in the animal experiments.

TABLE 1

The effect of glycine and urea ingestion, alone and together, upon creatinine-creatine excretion in man

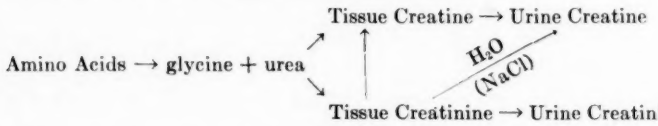
SUBJECT	DAYS OF EXPERIMENT	GLYCINE INGESTED	UREA INGESTED	EXTRA CREATININE			EXTRA CREATINE (AS CREATININE)		
				Theory	Excreted	Theory	Theory	Excreted	Theory
		grams	*grams*	*grams*	*grams*	*per cent*	*grams*	*grams*	*per cent*
P. P.	10	5		7.5	0.8	10.1	8.7	4.2	48.3
	14		5	9.4	2.2	23.4	10.9	3.3	30.3
H. H. B.	16	5		7.5	8.6	114.6	8.7	8.5	97.7
	18		5	9.4	11.6	123.4	10.9	6.8	62.4
Sum of theory, 7.5 + 9.4 + 8.7 + 10.9 = 36.5 grams creatine and creatinine Sum of extra excreted, 8.6 + 11.6 + 8.5 + 6.8 = 35.5 grams creatine and creatinine									
H. H. B.	34	5	5	16.9	21.2	125.4	19.6	13.4	63.3
Sum of theory, 16.9 + 19.6 = 36.5 grams creatine and creatinine Sum of extra excreted, 13.4 + 21.2 = 34.6 grams creatine and creatinine									

The results of the human experiments are very interesting (figs. 2 and 3). The theoretical amount of creatine and creatinine that could be formed from 5 grams of urea and 5 grams of glycine is 36.5 grams. Subject H. H. B. actually excreted 35.5 grams of extra creatine and creatinine in 2 weeks after ingestion of these amounts of glycine and urea separately (table 1). Results with subject P. P. were only about one-fourth as large as those of subject H. H. B. These results show that the human body can supply urea to combine with glycine, or glycine to combine with urea, in the synthesis of both creatine and creatinine when only one of them is ingested. Further proof of this theory would be afforded by a determination of the urea retention when glycine alone is ingested, a study which we shall begin in the near future.

When subject H. H. B. ingested 5 grams of urea and 5 grams of glycine together, 34.6 grams of extra creatine and creatinine were excreted, as compared to 36.5 grams required by theory.

These results are even more striking when it is considered that various factors, such as difference in absorption, excretion, storage and transformation into other products, can influence the synthesis of both creatine and creatinine under the experimental conditions specified. These factors, however, must have played little, if any, part in the transformations observed. Probably the extra glycine or urea, or creatine and creatinine formed from them, were simply stored in the muscles and the excess of the latter substances were then excreted into the urine at the rate of about 0.5 to 1.5 grams per day until all had been excreted.

In the case of H. H. B. the following scheme (based upon experimental evidence secured in this and other laboratories with the rat and man), is proposed to show the origin of both creatinine and creatine:[2]



If these results can be confirmed in other subjects, such a scheme would show that the amino acids are to be considered as the most logical precursors of both creatine and creatinine in body metabolism, a view we have often expressed. It should not be forgotten, however, that no extra creatinine was formed in the case of rats from urea or glycine alone, and only a slight amount from both together. Species differences, as well as differences within a given species, in creatine and creatinine metabolism may, and probably do, exist.

SUMMARY AND CONCLUSIONS

A stock diet, first alone, and later containing 5 per cent of urea, or 5 per cent of glycine, or 5 per cent of both, was fed to normal young rats, and the increases in muscle creatine and in creatine and creatinine excretion in the urine were observed. Similar observations were then carried out with double the amounts of urea and of glycine. Five grams of glycine, with and without 5 grams of urea, were ingested separately and then together by two adult subjects, and the increases in creatine and creatinine excretion in the urine observed. The following results were obtained:

1. In the rat the usual increases in muscle creatine were observed. Creatine synthesis was directly proportional to the concentration of glycine or urea fed separately or together in the diet.

[2] Tissue creatine was studied only in the animal experiments.

2. No increases in creatinine excretion were observed in the rat.

3. When 5 grams of glycine or 5 grams of urea were ingested separately by subject H. H. B., 97 per cent of the theoretical amounts of creatine and creatinine which could be formed from the specified amounts of these substances were excreted into the urine. When these amounts of glycine and urea were ingested together by this subject, 94.6 per cent of the theoretical values of creatine and creatinine were excreted.

4. Similar but much less striking results were obtained in subject P. P. under similar experimental conditions.

It may therefore be concluded:

1. When glycine is fed to rats, urea can be supplied for the creatine synthesis, and when urea is fed, glycine can be supplied for the same purpose.

2. When glycine is ingested by man, urea can be supplied for the synthesis of the guanidine group of both creatine and creatinine; when urea is ingested, glycine can be supplied for the synthesis of the acetic acid framework and methyl group of these substances.

3. It seems probable that in man, both creatine and creatinine can be synthesized from urea and glycine when they are ingested separately or together.

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THE RELATION OF BLOOD PRESSURE AND CONCENTRATION IN SERUM OF POTASSIUM, CALCIUM AND MAGNESIUM[1]

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In earlier studies the electrocardiographic changes appearing during the intravenous injection of potassium, calcium, and magnesium salts have been compared with the simultaneous concentrations of these ions in the serum (7, 12, 13). The present investigation deals with the relation between changes in blood pressure and the concentration of these ions in the serum. These are in turn correlated with the electrocardiographic changes occurring at comparable serum concentrations.

MATERIALS AND METHODS. Twenty-four dogs and eleven cats were used. The species and the nature of the anesthesia are indicated in table 1 which summarizes protocols of individual experiments. Artificial respiration was employed when necessary. Electrocardiograms were recorded at frequent intervals from lead II. Blood pressure was recorded by means of a mercury manometer connected to a cannula in the right femoral artery. KCl, $CaCl_2$, or $MgSO_4$ in isotonic solution were injected into the right femoral vein at a slow uniform rate until death. Injections were usually continuous; when intermittent, the fact is noted in the text. Frequent blood samples were withdrawn from the jugular vein or from the heart for analysis. Determinations of the concentrations of potassium, calcium, and magnesium in the serum were made by methods previously employed (7, 12, 13). Intermediate concentrations were determined by interpolation. In one series of experiments with magnesium the ureters were tied off prior to the experiment to prevent urinary excretion. Skin temperature changes following injection of magnesium and other ions were studied in another series using a Leeds and Northrup micro-max recording thermometer.

RESULTS. A. *Potassium and blood pressure.* Figure 1 shows the relation in five experiments between serum potassium and the mean arterial

[1] Aided by Grant no. 354 of the Committee on Therapeutic Research of the American Medical Association, and by grants from the Ella Sachs Plotz Fund and the Fluid Research Fund of the Yale University School of Medicine.

blood pressure during the slow intravenous injection of potassium. Injection was associated with a moderate increase in blood pressure never amounting to more than 20 mm. Hg. Alterations in blood pressure were minimal in the two experiments with complete absence of spontaneous fluctuatons in blood pressure prior to injection, and did not become evident before levels of serum potassium of about 10 m.Eq. per liter were attained. Analysis of associated electrocardiograms showed that the terminal fall in blood pressure coincided with the development of fairly advanced intraventricular block, preceding any significant alterations in heart rate. Very shortly after this the intraventricular block became more complete, and cardiac standstill supervened. After the appearance of intraventricular

TABLE 1

Summary of protocols of animals used

GROUP	SALT INJECTED	NUMBER OF ANIMALS	SPECIES	ANESTHETIC	WEIGHT		AMOUNT		TIME		RATE		CONC. OF CATION IN SERUM AT DEATH	
					Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.
					kg.	*kg.*	*cc.*	*cc.*	*min.*	*min.*	*cc./kgm./min.*	*cc./kgm./min.*	*m.eq. per liter*	*m.eq. per liter*
A	KCl	5	Dogs	Nembutal (4); morphine (1)	10	25	90	386	9	50	0.51	0.75	12.5	19.5
B	$CaCl_2$	5	Dogs	Nembutal (1); morphine (4)	6	22	11	460	11	75	0.57	3.17	43	169
C	$MgSO_4$	12	Dogs (5) Cats (7)	Local procaine (dogs); decerebrate (cats)	7	16	80	220	4	34	0.47	1.75	23	67
					2.3	2.5	12	66	3	47	0.38	1.60	22	61
D	$MgSO_4$	8	Dogs (4) Cats (4)	Nembutal	Ureters ligated; injections given intermittently									
E	$MgSO_4$ KCl $CaCl_2$ NaCl	5	Dogs	None	Skin temperatures									

block and the associated fall in blood pressure, the injection was interrupted temporarily in some experiments. In these instances prompt recovery occurred both in the electrocardiogram and in the blood pressure.

B. *Calcium and blood pressure.* Figure 2 shows the relation in five experiments between blood pressure and the level of calcium in the serum during the slow continuous intravenous injection of calcium. Blood pressure fell slightly in two of the five experiments during the first slowing phase (7). During the ensuing phase of increased heart rate a slight rise in blood pressure was observed in three of the five experiments. In one experiment blood pressure fell to zero when ventricular fibrillation developed, but rose again to its former level when a normal rhythm was reëstablished. Sudden fall in blood pressure to zero terminated the

experiments. Electrocardiograms showed arrest. In some experiments short periods of cardiac activity returned before final arrest, and blood pressures of 50 to 100 mm. Hg were recorded.

C. *Magnesium and blood pressure. Continuous injection* (fig. 3). Intravenous injection of this ion was invariably followed by an immediate fall in blood pressure, appearing at serum concentrations of magnesium from 2 to 5 m.Eq. per liter. This early fall in blood pressure thus preceded all neuromuscular and other cardiovascular manifestations. As the concentration was further increased, there was superimposed on the falling blood pressure a secondary rise, which occasionally even exceeded the control level. This rise was readily abolished by artificial respiration, and was in fact not seen in experiments in which artificial respiration was

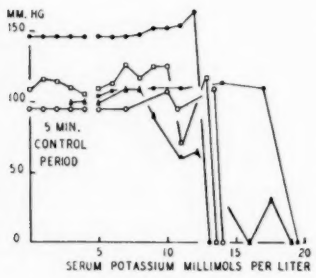

Fig. 1

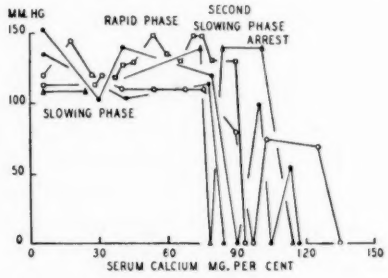

Fig. 2

Fig. 1. A graph showing the relation in 5 experiments (dogs) of mean arterial blood pressure in millimeters of mercury to the serum concentration of potassium.

Fig. 2. A graph showing the relation in 5 experiments (dogs) of the mean arterial blood pressure in millimeters of mercury to the serum concentration of calcium. The range of serum concentration at which the various electrocardiographic changes occurred is indicated. In one experiment (triangles) ventricular fibrillation occurred and blood pressure fell to zero. The heart recovered from fibrillation, blood pressure was reëstablished, and the animal finally succumbed to cardiac arrest.

maintained throughout the experiment. This elevation in blood pressure therefore represented an asphyxial rise, produced by the respiratory failure appearing at concentrations of 15 to 25 m.Eq. per liter. After the asphyxial rise had been abolished by artificial respiration blood pressure fell progressively to zero as the concentration of magnesium in the serum rose, except in a single experiment in which sudden cardiac arrest terminated the experiment at about the time of respiratory failure (12).

Serial electrocardiograms taken throughout the course of the experiments showed an initial increase in rate in the non-anesthetized animal, which was coincident with the early fall in blood pressure. The heart rate then gradually fell to its control rate or slightly below while the blood pressure dropped nearly to zero. Accompanying the change in heart rate was a

progressive and extreme P-R delay, with partial A-V block in the final stages, and a less marked intraventricular block (12). The heart quite suddenly stopped beating, usually about the time the diastolic blood pressure reached zero, although occasionally the heart continued to beat for as long as five minutes after this.

Relation of absolute concentration to blood pressure. In the experiments reported above the continuous injection of magnesium produced a continuously rising level of serum magnesium. Blood pressure fell progressively to zero, interrupted only in some experiments by an asphyxial rise due to respiratory failure. These experiments pointed to a definite relation between the level of serum magnesium and blood pressure. It could not, however, be determined from experiments of this type whether the

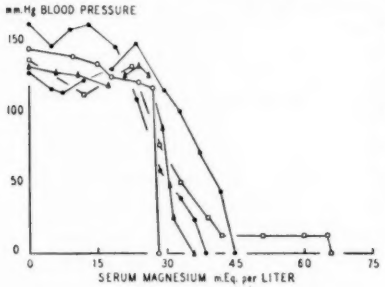

Fig. 3

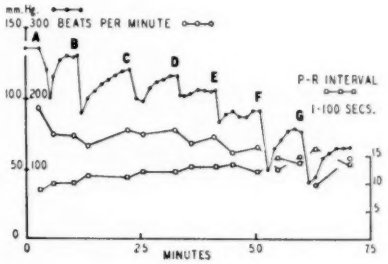

Fig. 4

Fig. 3. A graph showing the relation of the mean arterial blood pressure in millimeters of mercury to the serum concentration of magnesium in 5 experiments (dogs). In one experiment (open circles) the heart suddenly stopped beating at the time of respiratory arrest and blood pressure fell suddenly to zero. In all the rest the heart continued to beat, and blood pressure fell progressively to zero.

Fig. 4. Dog, ureters tied. Solid circles represent blood pressure in millimeters of mercury, open circles heart beats per minute, and open squares P-R intervals in 0.01 second. At *A*, *B*, *C*, *D* and *E* 30 cc. 0.154 M. $MgSO_4$ were injected intravenously. At *F* and *G* 60 cc. were injected.

drop in blood pressure was due to *change* in the concentration of magnesium in the serum, or whether there was a definite relation between the *absolute* level of serum magnesium and blood pressure. It was therefore necessary to ascertain whether the blood pressure would remain depressed, or whether it would return to its former level provided that the concentration of magnesium in the serum were kept constant. This was achieved by ligation of both ureters at the beginning of the experiment in order to suppress urinary excretion. Magnesium solution was then injected and time allowed for distribution through the extracellular fluids. At the end of a few minutes the concentration of magnesium attained a fairly constant level. A series of such separate injections was given, each of which raised the concentration in the serum by a certain increment. Determination

of magnesium in the serum was made at intervals. Artificial respiration was employed throughout to avoid asphyxial effects. Results are shown in figures 4, 5, and 6.

Blood pressure never returned completely to its control value after the first injection had been made, but it did tend to return to a point only a little below the preceding level after the injection of each increment (figs. 4 and 5). Typically each injection was followed by an abrupt drop, and by a short sharp rise. The blood pressure then rose more slowly over a period of 10 to 15 minutes, finally becoming stationary at a new level (figs. 4 and 5). With each subsequent injection the same sequence of events took place, and a new level was established at a lower blood pres-

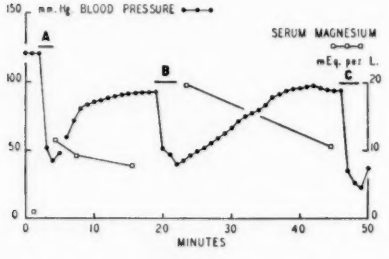

Fig. 5

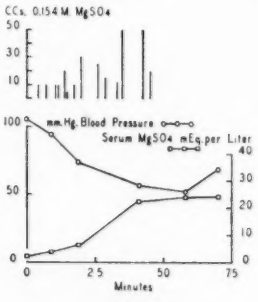

Fig. 6

Fig. 5. Dog, ureters tied. Solid circles represent blood pressure, open squares concentration of magnesium in the serum. At *A*, *B*, and *C* 100 cc. 0.154 M $MgSO_4$ were injected, the duration of the injection being indicated by the length of the horizontal line below each letter.

Fig. 6. Dog, ureters tied. Relation of blood pressure as shown by circles to concentration of magnesium in the serum (squares). Injections of 0.154 M. $MgSO_4$ indicated by vertical lines. Variations in blood pressure after each injection are not shown, but only the actual blood pressure at the time the blood sample was taken.

sure. In general recovery was less complete as the concentration rose above 15 m.Eq. per liter. Figures 5 and 6 show clearly, however, that no exact relation can be drawn between the level of blood pressure and the serum concentration. Heart rate and P-R interval were more exactly correlated with absolute concentration and were less affected by small changes in concentration (fig. 4).

Skin temperature. In order to determine whether or not the fall in blood pressure was associated with vasodilatation, skin temperatures were recorded in five unanesthetized dogs following the intravenous injection of magnesium (fig. 7). Small amounts of magnesium produced at once a marked rise in skin temperature which persisted for some time. Sodium, potassium, and calcium salts failed to produce any such effects.

Discussion. The continuous intravenous injection of calcium and potassium salts in isotonic solutions is found in these studies to have a relatively minor effect on the blood pressure throughout the ranges of concentration compatible with life. The terminal fall of blood pressure at death is clearly due to cardiac failure rather than to loss of the peripheral resistance. The absence of marked change in blood pressure until terminal cardiac failure from ventricular fibrillation or arrest following the injection of calcium is consistent with earlier studies. The sensation of warmth reported in human subjects after injection of calcium salts is probably due to stimulation of peripheral sense organs and not to vasodilatation (6, 8, 9). During the phase of marked cardiac slowing, recognized as the first effect of increased calcium (7), the blood pressure was observed to fall slightly in two experiments, while during the ensuing rapid phase, an equally small rise was noticed in three experiments. It is possible that

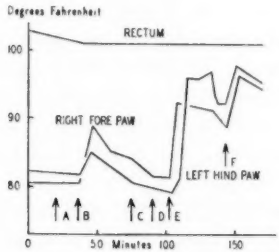

Fig. 7. Normal, unanesthetized dog. Arrows and letters indicate the intravenous injections of various substances, as follows: *A*, 50 cc. isotonic NaCl. *B*, 45 cc. isotonic $MgSO_4$. *C*, 45 cc. isotonic $CaCl_2$. *D*, 45 cc. isotonic KCl. *E*, 45 cc. isotonic $MgSO_4$. *F*, 1 mgm. acetyl-B-methyl choline chloride.

these alterations in blood pressure are direct reflections of changes in cardiac output. On the other hand the peripheral vascular system may be influenced in so far as the changes in heart rate may result from alterations in the activity of the autonomic nervous system.

Reports of the intravenous injection of potassium are less consistent. Mathison (10) reported a fall in blood pressure, while Camp and Higgins (3) observed a considerable elevation. It seems likely, as Mathison suggested, that the fall in blood pressure was due to cardiac failure, since potassium was given rapidly, and concentrations toxic to the heart might be reached almost at once. The mechanism of the abrupt rise observed by Camp and Higgins is not clear. It is known that in relatively concentrated solutions potassium salts are irritant when injected intravenously, and when administered directly will stimulate a variety of nervous and muscular tissues. It is generally recognized that intra-arterial injections

will evoke rises in blood pressure (10). When potassium is injected rapidly, local concentrations in the veins may reach levels high enough to cause a reflex rise in blood pressure. In our experiments, the salt was given more slowly, and concentrations sufficient to stimulate may not have been attained until shortly before the heart failed. Our experiments are on the whole in accord with those of Brewer and Larson (2) who failed to obtain increase in blood pressure when the level of serum potassium was elevated more slowly, although they did not continue the injection to the point of death. Our experiments support Brewer and Larson's contention that the blood pressure elevations produced by adrenalin cannot be due to an increase in serum potassium, since the average elevation of blood pressure observed at the extreme upper level of potassium consistent with life was only 15 mm. Hg.

The fall in blood pressure following the injection of magnesium is of an entirely different character. It begins almost at once and is marked before any of the other toxic effects of the ion are manifest. It is from the very beginning associated with intense cutaneous vasodilatation, as is apparent not only from the skin temperature experiments described in this paper, but from the flushing and subjective sensation of intense heat evoked whenever small quantities are injected in human subjects. Indeed this last effect is so constant that it has been used as a means of measuring the velocity of the circulation (1, 11, 14). That the vasodilatation is not confined to the skin is evident from the experiments of Haury who by a plethysmographic technique demonstrated a marked increase in the volume of the internal organs in animals and of the human leg after magnesium injection (4, 5).

It seems reasonable therefore to believe that vasodilatation plays an important rôle in the fall of blood pressure following magnesium. Whether or not there is some associated weakness of the cardiac contraction cannot be determined from experiments such as these. The early fall in pressure occurs at concentrations of magnesium which produce no electrocardiographic or other evidence of cardiac damage; it may also be antagonized by asphyxia which indicates at least that the heart is capable of maintaining a blood pressure of a normal height. It seems therefore unlikely that in the early stages cardiac weakness contributes significantly to the phenomenon. As the level of magnesium rises above 15 m.Eq. per liter heart block and other evidences of serious cardiac damage appear, and when the concentration rises high enough the heart stops beating altogether. It therefore is probable that toward the end deficiency of cardiac output may contribute to the fall in pressure.

It is difficult to decide whether the fall in blood pressure is wholly due to change in magnesium concentration or whether the absolute level is also of importance. Since the same blood pressure may be associated

with more than one level of magnesium in the serum, absolute concentration cannot be the sole factor. On the other hand the failure of the blood pressure to return each time to its full previous level suggests that absolute concentration of magnesium is a significant factor. This difficulty may to some extent be resolved by assuming *a*, that the presence of an excess of magnesium is a powerful vasodilating influence and that in general its activity is proportional to its absolute concentration, and *b*, that the cardiovascular mechanisms for maintenance of blood pressure can to a certain degree compensate for this influence, but that such compensation takes time and may not be wholly effective. Thus each new increment of magnesium even though small produces an immediate fall because of the delay in the action of the compensating mechanisms, yet since they do eventually come into play, the tendency each time is for the pressure to return toward the previous level. As the concentration of magnesium is raised higher and higher these compensatory forces are less and less effective in restoring the blood pressure to normal. Part of the recovery in blood pressure is also attributable to redistribution of magnesium through more of the body fluid, so that although no excretion takes place, a distinct drop occurs in the concentration in the serum in the short interval after each injection (fig. 5).

SUMMARY AND CONCLUSIONS

1. Potassium, calcium and magnesium salts were injected intravenously in dogs and cats at a slow uniform rate until death occurred.

2. The blood pressure showed only minor changes during the potassium and calcium injections until terminal cardiac failure supervened. There was then an abrupt fall to zero.

3. An immediate fall in blood pressure appeared when magnesium was injected. Artificial respiration being provided, this fall continued gradually and progressively until death.

4. Magnesium provoked an immediate rise in skin temperature, while potassium and calcium did not.

5. Magnesium is a powerful vasodilator while potassium and calcium, similarly administered, are without marked effect.

6. Vasodilator effect of magnesium is in part due to a change of the concentration of circulating magnesium and in part to the actual presence of an elevated concentration.

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THE DIODRAST CLEARANCE AND RENAL BLOOD FLOW IN NORMAL PREGNANT AND NON-PREGNANT WOMEN

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The diodrast clearance, under certain conditions, has been shown by Smith, Goldring and Chasis (1938) to be an indirect measure of the renal blood flow. When the plasma level of diodrast iodine is less than 5 mgm. per cent, the renal extraction ratio is close to 100 per cent, which suggests that all the diodrast is removed from the plasma which perfuses the excretory portion of the kidney. Since all of the blood diodrast is found in the plasma, the effective blood flow of the kidney may be obtained by dividing the plasma fraction into the diodrast clearance.

Smith, Goldring and Chasis (1938) and Chasis, Ranges, Goldring and Smith (1938) have reported the normal renal blood flow in men as averaging about 1340 ml. per 1.73 sq. m. of body surface per minute. For reasons detailed below, we consider this figure too high. We are, therefore, presenting our findings in nine normal non-pregnant women and eight normal pregnant subjects near term.

MATERIAL AND METHODS. Normal pregnant patients from the clinic were hospitalized for the tests. In a very few cases, patients who had been admitted for false labor were utilized. All normal pregnant patients were within four weeks of term. Non-pregnant normal patients were drawn from the post-partum and gynecological clinics, and were at least seven and usually more weeks post-partum. Three healthy non-pregnant nulligravidous women were also used.

A special catheter with six or seven openings was placed in the bladder. From here on the procedure described by Smith, Goldring and Chasis (1938) was followed. A continuous infusion of 10 per cent dextrose containing 1 ml. of 35 per cent diodrast per 100 ml. was given at the rate of 10 ml. per minute for 10 minutes; the rate of infusion was then cut down to 4 ml. per minute. About 40 minutes after the beginning of the infusion the bladder was washed out with saline, and the urine collection period begun. At this time the first blood specimen was taken (oxalated). Three urine collections were made, each over a period of about 45 minutes. The bladder was washed out twice each time with sterile saline. The washings

were combined with the urine for analysis. The second blood sample was taken midway in the test, and the third was obtained at the end.

Diodrast was analyzed as iodine, using a modification of Kendall's method as adapted by Smith, Goldring and Chasis (1938). The use of glass beads was omitted, and the samples of plasma and diluted urine were dried over night at 85 to 90°C. After fusion, the cooled melt was dissolved by adding water to the crucible and boiling carefully on a hot plate. The solution was poured directly into the analysis flask, and the crucible then rinsed six times with cold water brought to boil in the crucible. The final titration was made with 0.001 N sodium thiosulphate. All reagents were freed of iodine by the methods described by McCullagh (1934). Blank analyses were always negative. Frequent analyses of known solutions of both hippuran and potassium biniodate in urine and plasma gave results close to theoretical. All determinations were done in duplicate.

The plasma clearance of endogenous creatinine was simultaneously determined by the methods of Folin (Peters and Van Slyke, 1932). When the urine volume was in excess of 2 ml. per minute, the maximal urea clearance was measured by the manometric procedures of Van Slyke (Peters and Van Slyke, 1932).

For the determination of the plasma fraction, 1 ml. of well mixed blood was placed in a vaccine tube (designed by E. M. MacKay, Peters and Van Slyke, 1932), and centrifuged at 2500 r.p.m. for an hour. The volumes of cells and plasma were then read directly.

The effective renal blood flow was calculated by dividing the plasma clearance of diodrast by the plasma fraction. Hereafter, this figure will be called simply "renal blood flow." All clearances are corrected to a body surface area of 1.73 sq. m.

Results. In table 1 are shown the plasma clearances of diodrast, endogenous "creatinine" and urea, the hematocrits, and the calculated renal blood flows, "filtration fraction" and urea extraction ratios. The "filtration fraction" was calculated by dividing the clearance of apparent creatinine by the diodrast clearance (renal plasma flow). As indicated below, we do not regard the endogenous creatinine clearance as a measure of glomerular filtration. It is generally considered that about 40 per cent of the filtered urea is reabsorbed; as the data in table 1 show, the clearance of urea often exceeds that of creatinine. In no case does the clearance ratio of creatinine/urea approach 1.67. The "filtration fraction" shown is, therefore, much too low. This is unexpected, since Miller and Winkler (1938) found that the clearance of true endogenous creatinine is close to the level of glomerular filtration, and Miller and Dubos (1937) report that approximately 95 per cent of the apparent creatinine of the plasma is creatinine.

The renal extraction ratio for urea averages 8.6 per cent. This is close to the average of 8.7 obtained by calculation of the data of Landis, Elsom, Shiels and Bott (1936), discussed below. This figure also accords well

TABLE 1

Normal series of 17 patients

All data corrected to body surface area of 1.73 sq.m. Data given are averages of 3 clearance periods in non-pregnant patients, and of 6 clearance periods in pregnant subjects.

PLASMA CLEARANCE OF			HEMATOCRIT	"FILTRATION FRACTION"	UREA EXTRACTION RATIO	RENAL BLOOD FLOW
Diodrast	"Creatinine"	Urea				
Normal pregnancy, near term						
ml. per min.	*ml. per min.*	*ml. per min.*	*per cent*	*per cent*	*per cent*	*ml. per min.*
520	95.7	91.0	33.8	12.1	11.6	787
848	103.5	138.0	31.7	12.2	11.2	1233
560	66.5	64.2	29.5	11.8	8.6	793
513	69.7	47.5	26.0	13.6	6.9	694
607	67.5		31.7	11.1		886
672	101.5		32.5	15.1		995
593	64.3		29.0	10.8		833
568	63.3	82.8	30.0	11.1	10.2	812
Average 610	79.0	84.7	30.5	12.9	9.7	879
Non-pregnant normal						
461	55.5	49.4	38.8	12.0	6.6	752
531	56.5		33.0	10.7		792
542	57.6	48.1	36.5	10.6	5.7	851
485	60.8	71.0	37.0	12.5	9.3	769
601		61.0	42.8		5.8	1049
554	55.9	65.6	39.6	10.1	7.2	918
501	51.9	61.3	38.0	10.3	7.7	807
524	63.5	71.3	38.0	12.1	8.5	846
461	57.7		37.3	12.5		738
Average 518	57.4	61.1	37.9	11.3	7.3	836
Average of all 561	68.3	71.0	34.4	12.2	8.6	856

with the data obtained by simultaneous analyses of systemic arterial and renal venous bloods in dogs and rabbits (see Discussion).

There seems to be no effect of pregnancy upon the renal blood flow, despite the greatly increased cardiac output. The plasma clearance of diodrast is 18 per cent higher in the pregnant subject, but this is apparently explained by the hydremia of pregnancy—in each milliliter of whole

blood there is more plasma. The renal blood flow in this whole group ranges from 694 to 1233 ml. per 1.73 sq.m. per minute, and averages 856 ml.; the standard deviation is 126 ± 14.8 ml., and the standard error of the mean is 30.6 ml. These values for diodrast clearance and renal blood flow are considerably lower than the only published data. The discrepancy is discussed below.

Edwards and Biguria (1934) have demonstrated that intra-vascular injection of large doses of diodrast cause a transitory fall in the blood pressure. Although the amount and concentration of diodrast used in our measurements of the clearance are far below the doses used by Edwards and Biguria, or those used in routine pyelography, we have checked the blood pressures of many of our patients at intervals during the infusions. The average change in blood pressure was −1.3 mm. Hg in the systolic and +1 mm. Hg in the diastolic. About as many showed increases as had decreases in the blood pressure. The majority of patients did show variations of 10 mm. Hg or more.

Discussion. The data of Chasis, Ranges, Goldring and Smith (1938), comprising 34 diodrast clearances on 15 normal subjects, indicate an average renal blood flow of 1339 ml. per 1.73 sq.m. per minute. Since our average of 22 clearances (66 clearance periods) on 17 normal pregnant and non-pregnant patients is 856 ml. per 1.73 sq.m. per minute, the question arises as to which—if either—represents the physiologically normal renal blood flow.

The reliability of calculations of human renal blood flow from

$$\frac{\text{clearances in the human subject}}{\text{renal extraction ratios found in lower mammals}}$$

may be open to question. However such calculations give results not far from our average blood flow, determined by the diodrast clearance, as the data in table 2 show. Moreover, there are still further considerations to reinforce the argument for renal blood flows of about 800 to 900 ml./1.73 sq.m./minute.

The creatinine extraction ratios determined in other mammals can not be carried over to man, for in man there is apparently a tubular secretion of some creatinine. Such creatinine secretion does not occur in the dog.

The above calculations of renal blood flows for the human being, from human clearances divided by extraction ratios found in mammals, range from 690 to 1060 ml. per minute, and average 843 ml. per minute. Data from both dogs and rabbits have been used.

Again, on the basis of comparatively physiology, Smith (1937) in his book gives the average renal blood flows for the rabbit and dog as 3.2 and 3.3 ml. per gram of kidney per minute. (This is perhaps the fairest basis upon which to compare blood flows.) Our average blood flow of 860 ml.

per minute, calculated from the diodrast clearance, would give 3.3 ml. per gram of kidney per minute, in close agreement with the meager data from other mammals. The normal of 1340, reported by Chasis *et al.* (1938), would give 4.62 ml.

Another consideration which indicated that our renal blood flows are not too low is the low ratio of endogenous creatinine clearance/diodrast clearance. Since all of the apparent creatinine of the plasma either is not filtered or does not escape tubular reabsorption, the plasma clearance of "creatinine" is very probably lower than the level of glomerular filtra-

TABLE 2

Calculations of the blood flow through the human kidneys from average normal clearances in man divided by renal extraction ratios reported for lower mammals

AUTHOR	ANIMAL	SUBSTANCE INVESTIGATED	EXTRACTION RATIO	AVERAGE CLEARANCE IN MAN	CALCULATED RENAL BLOOD FLOW IN MAN
			per cent	*ml. per min.*	*ml. per min.*
Van Slyke, Rhoads, Hiller and Alving (1934)	Two-kidney dogs	Urea	10.5	75	715
	One-kidney dogs	Urea	8.7		862
Gordon, Alving, Kretzschmar and Alpert (1937)	One-kidney dogs	Urea	7.1		1,057
Sheehan (1932)	Rabbits	Urea	8.3		904
Weiss, Parker and Robb (1933)	One man	Urea	10.0		750
Landis, Elsom, Bott and Shiels (1936)*	17 cases of human renal disease	Urea	8.7		862
Van Slyke, Hiller and Miller (1935)	One-kidney dogs	Inulin	22.3	122	911†
Corcoran and Page (1939)	One-kidney dogs	Inulin	29.7		686†
Mean					843

* Calculated from average ratio between plasma clearances of urea and hippuran (0.145), and assuming average hematocrit of 40 per cent.

† Hematocrit assumed to be 40 per cent.

tion. This circumstance would make our "filtration fraction," of table 1, low. However, if the glomerular filtration in our normal subjects averaged 122.5 (which is 1.8 times our average creatinine clearance), the filtration fraction still would be less than 22 per cent—a not unreasonable figure, nor much above the average of 17.7 per cent found by Chasis *et al.* (1938), or the 20 per cent found by Van Slyke, Hiller and Miller (1935).

Finally a consideration of the cardiac output may be germane. Accepting Grollman's index of 2.2 liters per sq.m. per minute, the output in the ideal adult would be 3800 ml. per minute. Of this, 1000 ml. goes to the brain (Quastel, 1939). If 1340 ml. were to go to the kidney, only 36

per cent of the entire cardiac output would be left for all of the rest of the body. The average weight of the two kidneys together is 294 grams. In a 70 kgm. man, this would be 0.42 per cent of the body weight. A renal blood flow of 1340 ml. would require that 35.3 per cent of the cardiac output go to this small proportion of the body. (Our renal flow of 860 ml. would still require 22.5 per cent of the cardiac output.)

In recapitulation, the results of Smith and his collaborators are somewhat higher than might be expected from independent calculations of the sort made above. Diodrast itself, a diuretic and vaso-dilator, might conceivably cause some degree of renal hyperemia either in the kidney directly, or by increasing the cardiac output. Possibly our renal blood flow values are lower than Smith's because our infusions consisted only of diodrast in 10 per cent dextrose, while his consisted of diodrast, inulin, phenol red, and rarely sodium sulphate, in saline. Since the publication of Smith's work, Herrick, Mann and Sheehan (1939), using an electric thermostromuhr, have found that a single injection of phenol red increases the renal blood flow in unanesthetized dogs for about 10 minutes. Perhaps this explains the higher renal flow found by Smith *et al.*, since they gave continuous infusions, containing phenol red, before and during their clearance periods. Inulin, often markedly toxic, might have a similar effect. In this connection, it might be noted that Smith, Goldring, Chasis and Ranges (1938) have reported that the renal blood flow might be halved or doubled without affecting significantly the glomerular filtration. Yet the average glomerular filtration in normal patients whose renal blood flow they measured (Smith, Goldring and Chasis, 1938) was 137 ml. per minute, with all at or above the usual average of 122 ml. Does this perhaps indicate some degree of renal hyperemia?

Dr. Smith has called my attention to Wald's (1937) data which show that the average weight of the kidneys in 20 to 30 year old women is 257 grams. In Smith's normal group (all men, 30 to 40 years old), the mean kidney weight would be 323 grams, or 1.26 times that in our women. If we multiply our average normal by this ratio, we get an expected renal blood flow of 1080 ml. for men. Smith now has a series of 39 normal men, including 5 who have not been averaged in because of consistently low inulin clearances. The average renal blood flow for all of his cases is 1215 ml., only 13 per cent higher than the "expected" level calculated from our figures.

SUMMARY AND CONCLUSIONS

Diodrast clearances, as an indirect measurement of the renal blood flow, have been done on 9 normal non-pregnant women, and 8 normal pregnant women near term.

The average normal renal blood flow as calculated from the diodrast clearances averages 856 ml. per 1.73 sq. m. per minute, and ranges from 694 to 1233 ml. There is no effect of pregnancy upon the renal blood flow. Many data from comparative physiology are considered. Nearly all suggest a blood flow of about 800 ml. per minute for the human kidney.

The urea extraction ratio averages 8.7 per cent, as calculated from the plasma clearance of urea divided by the renal blood flow.

We wish to thank Drs. S. A. Cosgrove, J. F. Norton and E. G. Waters for their permission to use patients from their services in this study, and for reading and criticising the typescript. Many of the blood and urine urea determinations were done by Miss Frances Orsato and Mr. Peter Marotta.

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ADRENERGIC INHIBITION AT SYMPATHETIC SYNAPSES[1]

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Gasser (1937a, b) and Lorente de Nó (1938) have given us very satisfactory diagrams to explain activity at synapses in the cord and in the brain stem respectively. Transmission therein is regarded as largely electrical. Inhibition is attributed to the blockade at strategically placed internuncial neurons by stimuli converging upon them in suitably timed succession. According to this view, inhibition is but another aspect or sequela of excitation and no necessity is found for invoking a distinct, new process of an independent nature. Lloyd (1939) has adapted these diagrams in representing, also on an electrical basis, excitation and inhibition in the two neuron paths that characterize sympathetic ganglia.

However, the concept of a neurohumoral process continues to provoke deserved attention in any consideration of the mechanism of inhibition in the central nervous system or in its outlying portions, the sympathetic ganglia. Even Eccles (1937), one of the most astute and assiduous critics of the neurohumoral theory of synaptic transmission, grants acetylcholine the rôle of mediating long lasting excitation in the superior cervical sympathetic ganglion. It seems then that no picture of inhibition at sympathetic synapses can be complete without a further search for and evaluation of an inhibitory counterpart to acetylcholine, i.e., a humoral inhibitor. We are quite familiar with such an opposing regulatory play of humoral agents natural to the body, as with acetylcholine and adrenaline in the heart and, in fact, in almost all tissues innervated jointly by the sympathetic and parasympathetic divisions of the autonomic system.

In experiments on the influence of autonomomimetic drugs on sympathetic ganglia it became clear that adrenaline was capable of producing marked inhibition. Further work (Marrazzi, 1939b) in which doses of adrenaline down to 5 gammata per whole animal (3–4 kgm. cats and rabbits) were injected intravenously, showed that inhibition was invariably obtained without any evidence of preliminary stimulation, even when the smallest dose was used. The ganglionic action of adrenaline is not due to vasoconstriction, since it was clearly demonstrated that the ganglia

[1] Preliminary report presented at the annual meeting of the American Physiological Society, Toronto, April 1939.

are resistant to anemia, from complete obstruction of the circulation, for periods beyond that covered by the duration of inhibition. The effectiveness of the smallest quantities of adrenaline suggested that the action might be of physiological significance and the possibility of an adrenergic inhibitory mechanism in sympathetic ganglia was entertained (Marrazzi, 1939b, d).

It seemed of even greater interest to study the action of adrenaline liberated within the body. The present investigation, therefore, concerns itself with ganglionic inhibition produced by *a*, adrenaline liberated from the adrenal medulla, and by *b*, adrenaline or an adrenaline-like substance possibly accumulated, under the influence of ephedrine, at the endings of the preganglionic neurons entering the synapses. The relation of inhibition by adrenaline to the acetylcholine liberated at the synapse by preganglionic stimuli is examined.

METHODS. Changes in transmission in the superior cervical ganglion were followed by recording the response of ganglion cells to impulses traversing the synapses after being initiated in the preganglionic nerves by fixed, submaximal shocks. The action potentials in the postganglionic nerves serve as an admirable index of postsynaptic response.

Cats, anesthetized by the intraperitoneal injection of nembutal[2], 30 mgm. per kgm., were used. The lower portion of the superior cervical trunk was exposed and separated from the accompanying vagus and depressor nerves. It was sectioned at the base of the neck to isolate it from the central nervous system and the end was drawn on to chlorided silver stimulating electrodes housed in a small, glass, moist chamber. Submaximal shocks of constant intensity and duration were originated by a thyratron stimulator at the rate of 2 per second and delivered through an isolating transformer to the electrodes on the preganglionic trunk.

The postganglionic potentials were led by silver, silver chloride, woolen wick electrodes to a direct coupled amplifier actuating a Matthews oscillograph. The preparation was kept in good condition by conducting the dissection and experiment in a warm, moist atmosphere. The details of this technique and of the apparatus are described in a previous paper (Marrazzi, 1939a).

The left splanchnic nerve was sectioned under the diaphragm and isolated up to the semilunar ganglion without manipulating the adrenal. To liberate adrenaline from the adrenal medulla the nerve was stimulated with shielded electrodes at a frequency of 15/sec. for $1\frac{1}{2}$ to $2\frac{1}{2}$ minutes.

Ephedrine was injected intravenously to cause the accumulation of adrenaline or an adrenaline-like substance at the preganglionic nerve ends. The assumptions upon which this procedure was based are taken up in the discussion.

[2] Nembutal was kindly furnished by Abbot Laboratories.

RESULTS. The first columns in figures 1 and 2 present typical records obtained when the preganglionic, submaximal stimuli were adjusted to a level sufficiently above threshold (Erlanger, 1937) to yield a steady response. When by stimulating the nerve to the adrenal medulla adrenaline was liberated into the blood stream, there occurred a striking reduction,

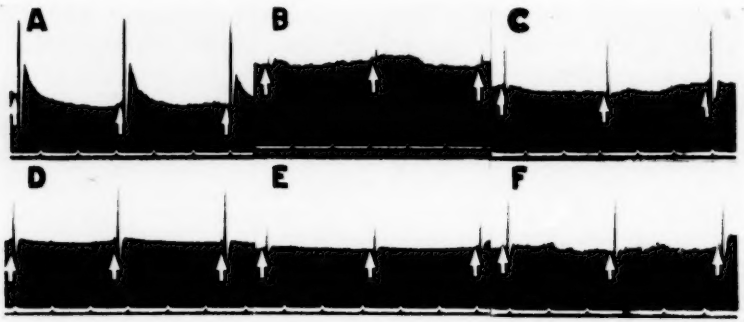

Fig. 1. Ganglionic inhibition by secretion from the animal's own adrenal. Sympathetic postganglionic potentials. Splanchnic nerve stimulation between 1st and 2nd columns. 1st column = controls; 2nd = maximum action; 3rd = recovery time in $\frac{1}{5}$ second. ↑ = stimulus artifact.

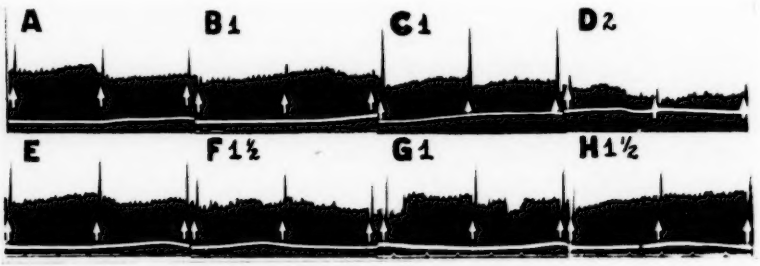

Fig. 2. Ganglionic inhibition overcome by increased stimulus. Sympathetic postganglionic potentials. Intravenous injection of adrenaline immediately after A; and of ephedrine immediately after E. 1st column = controls; 2nd = maximum action; 3rd = increased stimulus; 4th = original stimulus. Time in $\frac{1}{5}$ second. ↑ = stimulus artifact. Upper white line = respiration. Numerals on records indicate approximate time in minutes elapsed since previous record.

figure 1B, in the response to the unvarying test volleys presented to the ganglion. The heart was accelerated during the early part of stimulation but returned to a slower rate before stimulation ceased. With the destruction of adrenaline, recovery took place, figure 1C, and the experiment was repeated (D, E, F,) in the same animal. A marked depression

was again produced, figure 1E. The complete recovery that followed, F compared to D, is taken as evidence that depression of the ganglion from other causes had not intervened.

The intravenous injection of ephedrine, 10 mgm. per kgm., which may be considered to cause the accumulation of adrenaline or adrenaline-like substances at the preganglionic nerve ends (Burn, 1932; Gaddum, 1938), likewise depressed the ganglion though to a less marked degree, figure 2E – F.

The ganglionic inhibition described was not due to an increase in the effectiveness of acetylcholine at the synapses to the extent of producing block. This was clearly demonstrated when the acetylcholine thought to be released at the synapses was increased by strengthening the preganglionic stimuli. Instead of the further depression, which would be called for by the above hypothesis, the prolonged inhibition following a large dose of adrenaline, figure 2B, D, or following ephedrine, F, H, was readily broken through, C, G. The persisting depression on returning to the original stimulus intensity, figure 2D, H removes any suspicion that the "break through" was simply the result of the wearing off of the adrenaline or ephedrine.

Discussion. The presence of inhibition enduring over intervals as long as minutes (Rosenblueth, 1934) has naturally led to the proposal of a humoral hypothesis, which does not make demands as stringent as the nicety of timing in circuits of extreme complexity (Eccles, 1939; Lorente de Nó, 1938), which the concept of delay and reverberating pathways used in the electrical hypothesis requires.

At sympathetic synapses an excitatory humoral agent is known to operate. It possesses properties that make it a useful, if not indispensable, adjunct to the electrical theory of transmission. The absence of a humoral inhibitory agent, however, would constitute a serious gap. Although it is realized that final acceptance can only come from the actual detection of such an agent as it is liberated at the synapse, it is suggested that the previously described (Marrazzi, 1939b) ganglionic inhibitory action of adrenaline acquires added significance by the present demonstration of a corresponding action on the part of adrenaline physiologically originated. In favor of the actual operation of a humoral inhibitor in the superior cervical ganglion is the inhibitory action of adrenaline when it is injected or when it is liberated from the adrenals.[3] Moreover it is consistent with this concept that inhibition results when adrenaline or an adrenaline-like

[3] The ganglionic inhibitory action of circulating adrenaline serves homeostasis first by causing an initial vasodilatation of ganglionic origin; next by, as the amount increases, protecting the adrenaline activated effectors from subliminal postganglionic impulses thereby increasing the efficiency of these effectors; and finally by limiting excessive secretion by the adrenal medulla (Marrazzi, 1939 b, e).

substance is thought to be liberated at the synapses of the superior cervical ganglion. Thus the humoral inhibitory process at the sympathetic synapses would appear to be an adrenergic one.

A theory of adrenergic local humoral inhibition inevitably calls for the presence of specific inhibitory fibers or endings from which the mediator may be released. Inhibitory fibers have been suggested for the spinal cord by Kato (1934) and for the superior cervical ganglion by Eccles (1935a, b). Subsequently, Eccles (1937) has leaned away from his previous acceptance of inhibitory fibers in the cervical sympathetic trunk, but most recently (1939) cautions that "the absence of direct evidence of specific inhibitory synapses must not be taken to exclude their presence."

When tissues become innervated they acquire the property of reacting specifically to small doses of certain drugs. This rule, originally worked out for adrenaline (Elliot, 1905), has proved so reliable that reactivity to adrenaline in small doses, by itself leads to the inference that the effectors have been conditioned by special nerves (effector cells have developed receptors), i.e., that an adrenergic synapse exists. The action of adrenaline would therefore seem to warrant the assumption that adrenergic synapses exist between specific, inhibitory preganglionic fibers and ganglion cells.

Furthermore, the inhibitory action of ephedrine strongly suggests that adrenaline or an adrenaline-like substance is liberated from specific endings in the superior cervical ganglion. Current explanations of the sympathomimetic actions of ephedrine either attribute the major part of the action to a stimulation of sympathetic nerve ends (Burn, 1932) (with a consequent liberation of sympathin), or, by analogy with eserine, attribute it to an enzyme inhibiting effect (Blaschko et al., 1937) protecting adrenaline or an adrenaline-like substance (sympathin) from destruction (Gaddum, 1938; Gaddum and Kwiatkowski, 1938). In this instance the substance protected would be expected to come from preganglionic nerve ends. Either view necessitates the presence of specific adrenergic fibers.

The application of Gaddum's view to the superior cervical ganglion would imply more or less continued activity on the part of the postulated inhibitory preganglionic fibers. An observation may be adduced to indicate that this is likely. The asphyxiated ganglion exhibits a period of facilitation (Marrazzi, 1939b). Barcroft and Barron (1937) have noted that inhibitory mechanisms in the central nervous system are more susceptible to asphyxial depression than are the excitatory. Van Harreveld and Marmont (1939) attribute the asphyxial facilitation of spinal cord reflexes and tone, observed by themselves and by others, to a release of motor activity by just this differential effect. If this idea be extended to the synapses in the ganglia the deduction is obvious that inhibitory fibers exist and are active more or less constantly. Such fibers would perhaps have as one of their functions the restraint of ganglia from reverting to

the automaticity that Govaerts (1936) claims is rendered apparent by their decentralization.

The possibility that ephedrine exercises its influence on the ganglia by stimulating the adrenals has been eliminated by reproducing the effect in adrenalectomized animals (Marrazzi, in press, 1939e).

The idea has arisen, in various studies on the effects of adrenaline on striated muscle, that adrenaline might sensitize the muscle to acetylcholine (most recently, Luco, 1939) but this is not the manner in which it acts in these experiments (p. 741).

The ability of adrenaline to mobilize potassium directs inquiry toward this ion as a non-specific intermediary in bringing about the inhibition observed. However, stimulation precedes depression (superior cervical ganglion, Brown and Feldberg, 1935; stellate ganglion, Larrabee, Brink and Bronk, 1939). Even if a corresponding increase in potassium concentration in the ganglion were achieved by the small amounts of adrenaline that are effective, facilitation should precede inhibition, which is not the case in any of our numerous records. This makes it seem unlikely that potassium plays an inhibitory rôle directly related to that of adrenaline.

It seems justifiable, in the case of the superior cervical ganglion, to elaborate the existing concept of a transmission of "long duration," mediated by acetylcholine, to include a prolonged inhibition mediated by adrenaline or a closely allied substance. This would complete a ganglionic humoral system which could independently affect synaptic transmission. In addition, the quick, precision mechanism of electrical excitation and inhibition would be supplemented by the broader scoped activity of these humoral agents which may expand the temporal range of synaptic events at both ends of the scale—lowering the threshold to permit an earlier arrival at excitation or raising it to prolong inhibition.

SUMMARY

1. Synaptic transmission in the superior cervical ganglion is studied by recording the postganglionic action potentials in response to fixed preganglionic stimuli.

2. Synaptic inhibition is elicited by adrenaline liberated within the body.

3. Ephedrine injected intravenously exercises a similar though less marked effect.

4. In the light of the current theories of the way in which it acts, ephedrine is interpreted as causing, in these experiments, an accumulation of adrenaline or an adrenaline-like substance about the synaptic ends of preganglionic inhibitory fibers.

5. Since increasing the preganglionic stimulus, which is thought to increase the acetylcholine at the synapses, breaks through instead of enhancing adrenergic inhibition, this cannot be due to sensitization of the

ganglion cells to acetylcholine to the extent of causing the block known to result from an excess of this substance.

6. Adrenergic inhibition is discussed in relation to humoral and electrical transmission at sympathetic synapses.

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KETONE BODY-TOTAL CARBOHYDRATE UTILIZATION RATIOS AND THEIR RELATION TO THE PROBLEM OF KETOSIS[1]

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The fact that carbohydrates play an important rôle in the prevention of ketosis under various conditions has been verified abundantly both clinically and experimentally. It is, however, still a disputed question whether this rôle is fundamentally ketolytic or antiketogenic in nature since obviously ketosis may occur either as a result of deficient utilization (1) or increased production of ketone bodies (2) (3) (4).

We have recently (5) confirmed the observations of others (3) (6) that neither glucose nor ketones when injected intravenously affects the rate of utilization of the other. It seems obvious, however, that unless the total ketones being catabolized at any given time are in excess of the theoretical 2:1 ratio (1), relative to the carbohydrates already being oxidized, there need not be an increased utilization of the latter. Furthermore, the utilization of total body carbohydrates rather than that of blood glucose alone would be a truer criterion upon which to base a possible quantitative relation between the utilization of these two metabolites. We have, therefore, reinvestigated the problem by studying the utilization of both total body carbohydrate and total body ketones in normal and depancreatized dogs following the intravenous injection of sodium acetoacetate in varying amounts up to the tolerance of the animals. From the data obtained, the utilization ratios were calculated.

METHODS. Dogs after fasting 88 hours were anesthetized with nembutal (in a few cases with amytal) and the kidneys removed to prevent the excretion of the injected acetoacetate. In one group of animals pancreatectomy was performed on the fourth day preceding the experiments. Two types of preparations were employed: the eviscerated animal, prepared according to Markowitz *et al.* (7) and the nephrectomized animal with the

[1] These investigations were made with the financial aid of the Harry Snyder Research Fund, to the donors of which we wish to express our sincere thanks and appreciation. We are also indebted to Miss Gladys Garland for expert chemical assistance.

liver intact. These were allowed to rest for 30 minutes or more following the surgical manipulations for the purpose of equilibration and to overcome shock. The experiments lasted for two hours, in a few instances longer; body temperatures were maintained at the normal level. Sodium acetoacetate, freshly neutralized to a pH of 7.4, was injected intravenously; similar injections of glucose were also used in the eviscerated animals in order to maintain the blood sugar above the hypoglycemic level.

At the beginning and end of the experimental period, samples from two separate muscles and duplicate samples of liver were taken for glycogen determinations. The Good, Kramer and Somogyi (8) method was employed, the final glucose determinations being made by the Somogyi-Shaffer-Hartman method, using reagent 50 (9). Similarly, blood samples were taken from the femoral artery for quantitative determinations of glucose, lactic acid, non-protein nitrogen (N. P. N.) and total blood ketone bodies. Blood protein precipitation was accomplished by the zinc sulphate method in all cases. The other methods used were: for lactic acid, that of Friedemann and Graeser (10); for total ketone bodies, that of Behre and Benedict (11); for N.P.N., the micro-Kjeldahl, direct Nesslerization, method of Folin and Wu (12); and for blood sugar, that of Folin and Wu (13). The Shaffer-Hartman blood sugar method is unreliable in the presence of an excess of ketone bodies.

In order that one may calculate, from the blood constituents alone, the total utilization of glucose as such, the equivalents of glucose represented by the N.P.N. and lactic acid, and the total ketones remaining unutilized at the end of the experiment, it was necessary to take into consideration not only the blood volume, but also that of the fluid of the lymph and tissues into which there was presumably a diffusion equilibrium with the blood plasma. The distributions of glucose and total ketones in this sense were determined by two methods. One of these has already been reported (14), the other was similar to that employed by Burn and Dale (15). The two methods gave almost identical values. It was assumed that lactic acid and N.P.N. were distributed to the same extent as glucose.

From a rather large series of determinations, using the two methods, we have adopted the following distribution values: for eviscerated dogs—glucose, lactic acid and N.P.N. each 25 per cent and total ketone bodies 40 per cent of the original body weight; for non-eviscerated-nephrectomized dogs, the corresponding values were 33.3 per cent and 50 per cent respectively. Muscle was calculated as 50 per cent, liver as 3 per cent of the body weight.

The total carbohydrate utilization, expressed as glucose per kgm. per hour, was calculated in a manner similar to that of Soskin and Levine (16); the total ketone body utilization, expressed as acetone per kgm. per hour, was determined by subtracting the total ketones present at the end

of the experiment from those injected. The following is an example of such a calculation:

	Average change mgm. per cent per hour	*Glucose equivalent mgm., kgm., hour*
Muscle glycogen	32	160
Liver glycogen	433	130
Blood N.P.N.	3	36
Blood glucose	13.2	44
Total		370
Blood lactic acid	26.4	88
Net total glucose utilization		282

$\frac{282}{180}$ = 1.57 millimoles (mM) per kgm., hour of total carbohydrate utilized.

	mgm., kgm.
Acetoacetate injected (expressed as acetone)	2,316
Blood total acetone rise at end of experiment = 270 mgm. per cent; the equivalent of unutilized ketones	1,350
Ketones utilized in two hours	966

$\frac{966}{2 \times 58}$ = 8.3 mM per kgm. per hour. Therefore:

$$\frac{8.3 \text{ mM total ketones}}{1.57 \text{ mM total glucose}} = 5.3 \text{ utilization ratio}$$

The calculations for the eviscerated animals were similar to the above with the exception that determinations of liver glycogen and blood N.P.N. were omitted. Gluconeogenesis from protein was calculated from the blood N.P.N. on the assumption that 58 per cent of the former was converted into glucose in the liver. The increase in lactic acid represented an equivalent of carbohydrate not utilized and therefore was deducted.

RESULTS AND DISCUSSION. The essential data obtained from 9 normal, 7 eviscerated and 10 depancreatized dogs are presented in table 1. An analysis of these reveals several fundamental facts.

The utilization of ketone bodies is proportional to their own concentrations in the blood and tissues as indicated by the quantities of sodium acetoacetate injected. This relationship is illustrated graphically in figure 1. On the other hand, their utilization bears no relationship to the blood sugar level and is quite independent of the consumption of blood sugar or total body carbohydrate. Ketone body-glucose utilization ratios varying from 0.7 to 19.6 were obtained. Again there is no correlation between these and carbohydrate consumption. However, an approximately linear relationship does exist between these ratios and the utilization of ketone bodies. Ratios below the theoretical 2:1 were obtained when their concentration and utilization were low, higher ones when they were high and approximately theoretical ratios occurred only with intermediate values. This relationship existed whether the animals were eviscerated, non-eviscerated or depancreatized.

These observations are incompatible with the theory that a rather definite quantitative stoicheiometric relationship exists between the oxidation of ketone bodies and glucose (1). If such a relationship did prevail, these ratios should never exceed significantly the theoretical 2:1 and, when the ketone bodies are in excess, the rate of their utilization should be determined by the level of carbohydrate consumption and not by their own concentrations. Furthermore, since neither the injection of glucose nor the injection of excess ketone bodies affects, in the least, the rate of oxidation of the other, the existence of such a quantitative relationship seems to be extremely doubtful.

TABLE 1

Utilization ratios of total carbohydrates and total ketones

N = Normal nephrectomized dogs
E = Eviscerated nephrectomized dogs
DP = Depancreatized nephrectomized dogs

DOG	WEIGHT	AVERAGE BLOOD GLUCOSE	Na ACETOACETATE INJECTED (AS ACETONE)	TOTAL CARBOHYDRATE UTILIZATION (AS GLUCOSE)		Na ACETOACETATE UTILIZATION (AS ACETONE)		RATIO mM Ac. / mM Gl.
	kgm.	*mgm. per cent*	*mgm. per kgm. hr.*	*mgm. per kgm. hr.*	*mM per kgm. hr.*	*mgm. per kgm. hr.*	*mM per kgm. hr.*	
N1	8.5	84	150	164	0.91	144	2.48	2.6
N2	11.0	53	230	272	1.51	222	3.84	2.5
N3	7.5	67	356	205	1.14	247	4.26	3.7
N4	7.4	65	582	341	1.89	283	4.88	2.6
N5	13.6	60	618	239	1.33	304	5.24	3.9
N6	14.8	63	1,016	233	1.29	641	11.40	8.9
N7	6.8	65	1,158	318	1.77	483	8.30	4.7
N8	10.5	50	1,400	387	2.15	658	11.30	5.2
N9	13.7	56	1,456	429	2.38	756	13.00	5.4
E1	14.5	171	293	432	2.40	210	3.62	1.6
E2	14.9	194	346	422	2.34	181	3.12	1.3
E3	15.9	263	366	354	1.97	235	4.10	2.1
E4	14.7	176	383	413	2.29	259	4.47	1.9
E5	12.5	200	589	401	2.23	432	7.45	3.3
E6	12.0	52	722	489	2.73	605	10.40	3.8
E7	11.5	94	807	459	2.55	728	12.60	4.9
DP1	12.0	311	100	365	2.03	80	1.38	0.7
DP2	11.0	283	223	382	2.12	127	2.19	1.0
DP3	7.5	315	267	229	1.27	120	2.07	1.7
DP4	5.5	240	288	100	0.55	175	3.02	5.5
DP5	19.2	213	1,032	238	1.32	476	8.20	6.2
DP6	15.5	256	1,065	381	2.14	530	9.14	4.3
DP7	19.0	242	1,143	89	0.50	568	9.80	19.6
DP8	15.0	259	1,265	467	2.59	618	10.65	4.1
DP9	6.7	297	1,313	414	2.30	644	11.10	4.8
DP10	16.5	321	1,333	550	3.00	547	9.43	3.1

In the light of the "non-utilization" theory of diabetes the existing

ketosis is assumed to be the result of a deficient ketone body utilization in the tissues incident to a reduced capacity to oxidize carbohydrates in the absence of insulin. Contrary to this point of view, our data show that pancreatectomy does not affect significantly the rate of ketone body utilization or the so-called ketolytic ratios. These ketone body utilization values for diabetic animals of four days' standing and the corresponding utilization ratios as well are undoubtedly too low since they are based on the injected or exogenous ketones only. When the calculations are made on exogenous plus endogenous ketone bodies, as previously shown by Chaikoff and Soskin (2), these values fall within the normal range. We

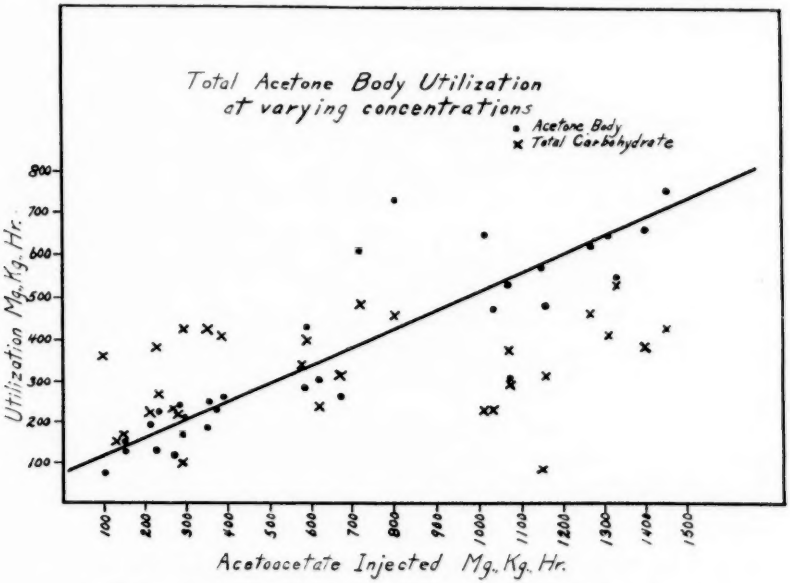

Fig. 1. Utilization of total ketone bodies and total carbohydrate at varying acetoacetate concentrations.

have confirmed the observations of these investigators that the abdominal viscera, of which the liver is most significant in the present discussion, do not utilize appreciable quantities of ketone bodies since evisceration does not reduce the utilization values. Our results also confirm the observations of Soskin and Levine (16) that the consumption of carbohydrates by the diabetic dog at its characteristic blood sugar level may be as high or even higher than that of the normal animal at its characteristic blood sugar level.

The evidence presented in this paper supports the conclusion that the action of carbohydrates in the relief or prevention of ketosis is not ketolytic

in nature; in fact, that this effect is not in the extrahepatic tissues at all. Evidence is now available which shows that the tissues are capable of utilizing both carbohydrates and ketone bodies in all conditions in which ketosis is known to occur (4) (16), and under these same conditions the available carbohydrate, except for liver glycogen, exists at practically normal levels. By the exclusion of an extrahepatic origin of ketosis and seat of "antiketosis," the present evidence supports the "over-production" theory. According to this the primary if not the only site of ketone body production is in the liver (2) (4) and the antiketogenic action of insulin, glucose and other glycogen formers is one of inhibiting the production of these bodies by this organ (2) (3) (4) (18), possibly through a mechanism involving the anterior lobe of the pituitary (4).

SUMMARY

Utilization ratios for total ketone body and total carbohydrate have been determined on eviscerated, nephrectomized-normal and depancreatized dogs following the intravenous injection of sodium acetoacetate in amounts up to the tolerance of the animals. Ratios significantly higher than the theoretical 2:1 prevailed when the ketone body concentrations were high, lower ones when these were low. They were not affected significantly by pancreatectomy or evisceration.

The rate of ketone body utilization by the tissues is not influenced by the blood sugar level, the utilization of total carbohydrates, evisceration or pancreatectomy, but by their own concentrations in the blood and tissues.

The existing evidence for and against the ketolytic and antiketogenic theories of carbohydrate action in relieving ketosis is briefly discussed. The antiketogenic theory is favored.

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EFFECT OF MARE SERUM HORMONE ON THE MALE AND FEMALE MOUSE

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The mouse has been shown to respond to mare serum hormone by ovarian weight increase in the immature female (1, 2, 3, 4). Hamburger (2) and Moricard and Gothie (5) have demonstrated that follicular development occurred with low doses of this extract administered in 4 or 5 day treatments whereas Hamburger (2) also induced luteinization in this period of time with higher doses.

These experiments extend the consideration of the effects of mare serum hormone to include the mature and immature male and also the mature female mouse. The effect of identical hormonal treatment on the mature and immature animals of both sexes was considered.

MATERIALS AND METHODS. In these experiments, 111 albino mice (Hygenic Strain) were employed, of which 43 served as litter-mate controls. Immature, mature and castrate male and female animals were injected with 0.5 r.u. (Cartland-Nelson) of mare serum hormone daily for 10 days unless otherwise stated. All mice were sacrificed the day following cessation of treatment. The weights of the ovaries, testes and combined seminal vesicles and prostates were taken at autopsy. Histological sections of the ovaries, uteri, vaginae, testes, seminal vesicles and prostates were prepared. Ovaries were sectioned serially.

Mare serum hormone "Gonadogen" was supplied through the courtesy of Dr. G. F. Cartland of the Upjohn Company, to whom the writers are greatly indebted.

RESULTS. Thirteen immature female mice, 12 to 18 days of age, were injected and a marked ovarian weight increase resulted. Twelve day old mice responded with as much as a 9-fold-weight increase of the ovaries. Control ovaries averaged 3.2 mgm. whereas the stimulated ovaries varied from 21 to 29 mgm. A varied histological condition was observed. The ovaries of litter-mate controls were composed entirely of small follicles and in most cases antrum formation was absent (fig. 3). The stimulated ovaries exhibited good sized follicles, usually of uniform size, and the oc-

casional presence of a hemorrhagic follicle. Although all animals received the same treatment, 2 mice exhibited ovaries composed entirely of stimulated follicles (fig. 4) but in all other cases lutein tissue predominated, much of which was corpora lutea atretica (fig. 5).

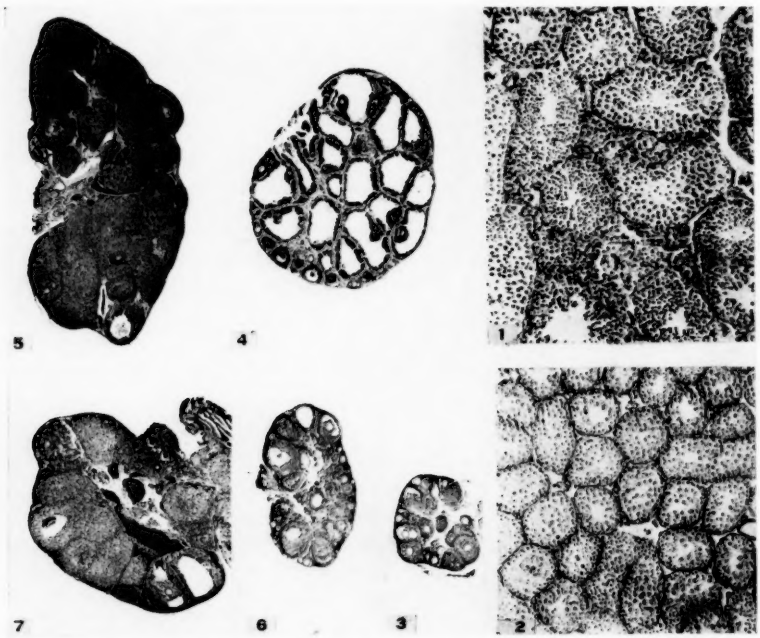

Fig. 1. Testis of mouse after mare serum hormone treatment. Sperm heads absent. Interstitial tissue increased. Twenty-two days of age at autopsy. × 140.

Fig. 2. Control testis. Twenty-two days of age at autopsy. × 140.

Fig. 3. Ovary of 22 day old control. × 50.

Fig. 4. Ovary showing only follicle stimulation after treatment. Twenty-two days of age at autopsy. × 50.

Fig. 5. Ovary showing only corpora lutea after mare serum hormone. Twenty-two days of age at autopsy. × 50.

Fig. 6. Mature control ovary. × 50.

Fig. 7. Mature ovary stimulated by mare serum hormone showing the predominance of lutein tissue. × 50.

General stimulation of the uterus was also observed. The vaginae were cornified in some instances whereas an infiltration of leucocytes was observed in other animals. These results confirm previous investigations (2, 5) and serve to establish the potency of the preparation used.

Thirteen young adult female mice, 34 to 41 days of age, were treated and

as much as a 6-fold ovarian weight increase resulted. In no instance were ovaries consisting only of stimulated follicles observed. Although some follicles were usually present, lutein tissue predominated (figs. 6, 7). One litter of animals received 7.5 units in 15 days, but the weight response was less pronounced. Injection of 0.5 r.u. daily into 78 day old female mice for 6 days produced an average 3-fold ovarian weight increase. Control ovaries averaged 9.4 mgm. as compared to an average of 26.7 mgm. for the ovaries of the injected animals. However ovarian weight varied between 19 and 38 mgm. Corpora lutea were predominant in these ovaries as with the mice receiving longer treatment.

In view of the pronounced influence on the ovary of 5 r.u. of mare serum hormone administered in 10 days it appeared of interest to employ the same treatment to test the effect on the male. Ten immature males, 12 to 14 days of age, were injected but the testis weight was not increased over that of the controls, both groups averaging 64 mgm. However, an increase in interstitial tissue occurred and a corresponding weight increase of the accessory organs resulted. The combined seminal vesicle-prostate weight averaged 88 mgm. as compared to 17 mgm. for the controls. Spermatogenesis was not hastened as determined by the absence of sperm head in the controls and in 9 of the 10 experimental, although one animal exhibited a few sperm head. However, increase in the number of mitoses and increase in tubular size was evident as judged by examination (figs. 1, 2). Eight 18 day old males received the same treatment with no resulting alteration in testis weight or hastening of spermatogenesis. Interstitial tissue was increased in most cases, a condition which was reflected in the average 5-fold weight increase of the accessory sexual organs. In contrast to the results with the immature males, eight young adult males, 32 to 40 days of age exhibited little or no increase in interstitial tissue and correspondingly there was no effect on testis weight and no apparent effect on spermatogenesis.

Nine castrate males and 7 ovariectomized female mice showed no response in the accessory sexual organs to mare serum hormone.

Discussion. Mare serum hormone has been shown by other investigators to produce much the same reaction in immature female mice as in rats. Cole (6) reported little effect on follicular maturation in 10 day old female rats with this extract. In these experiments it was found that a 12 day old female mouse responded more markedly as to ovarian weight increase than adult females when both were subjected to the same treatment. Follicular maturation resulted as well as the formation of corpora lutea. In two immature mice the ovaries consisted entirely of stimulated follicles whereas in other cases lutea tissue was predominant.

Cole (6) and Moore (7) have shown that the immature male rat would respond to injections of mare serum hormone with an increase in testis

weight, an increase in interstitial tissue and a corresponding weight increase of the accessory sexual organs, but hastening of spermatogenesis did not occur. The effect corresponded with the dose administered and the time period injected as small doses had little effect. Using the same dosage which produced a marked response in female mice it was found that testis weight of immature male mice remained unaltered. However, the interstitial tissue did increase with an increase in the accessory organs. Twelve day old males exhibited a good response. Spermatogenesis was not hastened as sperm heads were absent in contol and experimental animals. Possibly larger doses or longer treatment would produce an increase in the weight of the testes of mice.

The mature male rat showed no marked changes as to testis weight or histology when injected with this hormone (8) and similiar results were obtained with mature male mice.

SUMMARY

Mare serum hormone has been shown to be capable of producing a stimulating effect on the gonads of 12 day old male and female mice. Ovarian weight increase resulted in mature and immature female mice. Follicular maturation was induced with a few cases of only follicle stimulation but the formation of lutein tissue was predominant. Testis weights remained unaltered in all males, but an increase of the interstitial tissue occurred in immature males with a resulting increase in the weight of the accessory sexual organs. Hastening of spermatogenesis to the sperm head stage was not observed.

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THE RELATION OF LIPOCAIC TO THE BLOOD AND LIVER LIPIDS OF DEPANCREATIZED DOGS[1]

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Alterations in the concentration of fat in the blood and livers of depancreatized dogs have been noted by various observers. There is not however complete uniformity of opinion regarding the nature of the changes observed. In diabetes mellitus in man it is generally agreed that when a deviation from the normal occurs in the blood it is in the direction of hyperlipemia. The situation in the depancreatized dog is complicated by the fact that the absence of pancreatic juice in the intestine decreases the absorption of fat and thus automatically places the animal on a low fat diet. Allen (1922) reported that severely diabetic dogs having only a remnant of pancreas along the duct regularly develop hyperlipemia of varying degree. Himwich (1932) also found that the blood fat in depancreatized dogs is higher than normal. On the other hand Chaikoff and Kaplan (1934, 1935) reported a decrease in all the lipid constituents of the blood shortly after pancreatectomy and that the administration of raw pancreas raised these to higher than normal values.

It is the purpose of this paper to report the alterations that we have found to occur in the total serum and liver lipids of dogs following pancreatectomy and the subsequent administration of lipocaic (Dragstedt, Van Prohaska and Harms, 1936).

EXPERIMENTAL PROCEDURE. Pancreatectomy has been performed under general ether anesthesia; biopsy of the liver occasionally with the aid of local infiltration with novocaine but without ether. Following operation the animals have been placed on a diet of bread, beef, and whole milk plus vitamine supplements in the form of brewer's yeast and cod liver oil. Regular and protamine-zinc insulin have been administered in amounts sufficient to keep the sugar excretion less than 10 grams per day. The total lipids of the serum were estimated gravimetrically as follows: Three

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cubic centimeters of serum, carefully measured, were run gradually and with continuous rotary agitation into 60 to 70 cc. of 3 to 1 alcohol-ether in an extraction flask. The material was boiled for an hour under a reflux condenser and filtered hot into a 125 cc. Erlenmeyer flask. The precipitate was washed with small portions of boiling alcohol-ether and the solvent was then blown off with an electric fan. The residue was moistened with 3 to 5 cc. of water, acidified with a drop of concentrated HCl, and extracted repeatedly with petroleum ether. The petroleum ether extracts were transferred quantitatively to tared flasks and the petroleum ether blown off. The residues were allowed to stand several hours in the balance room and were then weighed. The values so obtained represent all the material soluble in alcohol-ether and petroleum ether and are higher than those obtained by most oxidation methods. All the solvents used were redistilled and left no appreciable residue on evaporation.

TABLE 1

Serum lipid values, in per cent of preoperative levels, of twelve dogs receiving insulin but no lipocaic during the first few weeks following pancreatectomy

The preoperative levels ranged from 690 mgm. to 1080 mgm. (average, 876 mgm.) per 100 cc.

WEEKS AFTER PANCREATECTOMY	NUMBER OF DETERMINATIONS	RANGE OF MINIMUM VALUES	RANGE OF MAXIMUM VALUES	AVERAGE OF ALL VALUES
		per cent	*per cent*	*per cent*
1st	36	74–139	116–161	122
2nd	23	68–104	73–104	87
3rd	17	34–106	41–113	79
4th	18	27–101	49–111	74
5th	12	24–84	37–87	63
6th	10	26–65	42–68	55

We have reported changes in total serum lipids almost exclusively (a few representative cholesterol values are given in table 3) because we have observed that these are more extensive and consistent than those of any other component. Cholesterol was estimated by Shapiro's modification of Schoenheimer and Sperry's method.

RESULTS. The data represented in table 1 indicated that whereas marked variations in the blood lipids of individual animals takes place following pancreatectomy the general trend is quite definite. During the first week after the operation the serum lipids are usually found to be above normal. This hyperlipemia may be quite marked in fat animals and especially so if insufficient amounts of insulin are given. During the second week however the lipids usually return to normal values and from then on a progressive decline takes place so that after five or six weeks the total lipid level is about one-half the normal value.

This decrease in the concentration of blood lipids is accompanied by a deposition of fat in the liver, as indicated in table 2. The data represented in table 3 indicate that fluctuations in blood cholesterol occur similar in nature to the total lipids, but most pronounced in the cholesterol ester fraction. Biopsy of the liver 36 days after pancreatectomy, at a time when the lipid constituents of the blood had reached a very low level, revealed a marked fatty infiltration as determined by both histological and chemical examination of representative areas.

TABLE 2

Total blood lipid, blood sugar, and liver changes which occurred in four depancreatized dogs receiving insulin but no lipocaic during the first 23 days following pancreatectomy

	DAYS AFTER PANCREATECTOMY					
	0	1	3	9	16	23
Dog 1						
Blood sugar, mgm. %		102	140	121	286	293
Blood lipids, mgm. %	690	960	1,020	590	710	660
Liver lipids, % wet wt.	5.6%					16.1%
Dog 2						
Blood sugar, mgm. %		151	234	204	260	347
Blood lipids, mgm. %	870	930	1,170	640	730	580
Liver lipids, % wet wt.	7.5%					28.9%
Dog 3						
Blood sugar, mgm. %		94	212	208	241	260
Blood lipids, mgm. %	850	1,180	1,330	740	1,170	750
Liver lipids, % wet wt.	4.6%					19.5%
Dog 4						
Blood sugar, mgm. %		113	125	208	264	264
Blood lipids, mgm. %	710	1,140	970	630	670	550
Liver lipids, % wet wt.	4.3%					23.2%

The oral administration of lipocaic to the insulin treated depancreatized dog, at a time when the blood lipids have reached a low level and biopsy reveals a marked accumulation of fat in the liver, produces a prompt rise in the serum lipids often to levels considerably exceeding the preoperative value. If a biopsy has been performed, a temporary rise in lipid level due to the operation must be taken into account and the administration of lipocaic deferred until this effect is over. After the initial hyperlipemia produced by the lipocaic, the lipid level tends to fluctuate about the preoperative value. Table 4 shows the effect of 4 weeks of lipocaic therapy on the blood and liver lipids of three of the four dogs mentioned in table 2. As will be noted, the restoration of the serum lipids occurs promptly, as do

the relief of symptoms and the improvement in liver function, but the recession of fat from the liver is a much slower process.

DISCUSSION. Several hundred estimations of the total blood lipids in approximately fifty depancreatized dogs have been made. Whereas marked variations in individual animals have been found, certain general

TABLE 3

Total serum lipids and free and total cholesterol levels of four depancreatized dogs given insulin but no lipocaic for the first 36 days following pancreatectomy

	DAYS AFTER PANCREATECTOMY							
	0	1	3	9	16	23	30	37
Dog 5								
Total lipids	1,040		1,260	1,080	582	437	314	385
Free cholesterol	66	89	70	52	57	66	55	48
Total cholesterol	172	282	211	160	185	165	121	102
Dog 6								
Total lipids	900	1,100	1,230	910	870	980	680	610
Free cholesterol	65	66	92	52	54	50	51	53
Total cholesterol	150	217	250	159	120	109	129	121
Dog 7								
Total lipids	920	1,230	1,040	770	740	930	670	550
Free cholesterol	61	77	70	51	52	50	55	52
Total cholesterol	164	226	230	154	122	117	132	108
Dog 8								
Total lipids	820	970	640	610	930	670	480	530
Free cholesterol	70	71	80	59	49	49	48	45
Total cholesterol	170	191	205	203	166	123	114	103

Biopsy results by two methods performed independently

DOG	HISTOLOGICAL	CHEMICAL
		per cent
5	5+	42.0
6	3+	12.9
7	5+	35.2
8	5+	38.8

trends have been recognized. In normal dogs the total serum lipids were found to vary between 700 and 1050 mgm. per 100 cc. with an average of about 850. During the first week after pancreatectomy but with adequate insulin therapy, the serum lipids were found to be elevated approximately 25 per cent above the normal. In fat animals a lipemia of 2 to 3 per cent

was occasionally found. The withdrawal or inadequate administration of insulin during the first week after pancreatectomy usually caused a marked hyperlipemia and occasionally acidosis which promptly disappeared upon resumption of insulin. Beginning with the second week after pancreatectomy and with adequate insulin therapy the total serum lipids, and the free and total cholesterol, gradually decreased. After five or six weeks, the total lipids fell to approximately half the normal value. At this time the animals commonly displayed the usual symptoms and findings of fatty liver due to lipocaic deficiency, viz.: decrease in glucose excretion, decrease in insulin requirement and tolerance, impairment in liver function as determined by the bromsulphalein test, and loss of strength and appetite. Examination by biopsy revealed the liver to contain 30 to 40 per cent of

TABLE 4

Changes effected by lipocaic therapy in three insulin treated depancreatized dogs having fatty livers

Blood lipids in mgm./100 cc.; liver lipids in per cent wet weight.

	PRE-OPERATIVE	WEEKS ON LIPOCAIC				
		0	1	2	3	4
Dog 1						
Blood	690	660	830	704	680	720
Liver	5.6%	16.1%				7.6%
Dog 2						
Blood	870	580	830	780	670	860
Liver	7.5%	28.9%				15.0%
Dog 4						
Blood	710	550	870	1,120	520	510
Liver	4.3%	23.2%				18.5%

fat. This fat was not removed from the liver by the administration of insulin, in sharp contrast to the fatty infiltration which occurs within the first week after pancreatectomy when no insulin is given.

The oral administration of lipocaic to such animals produced a definite elevation of the total serum lipids usually within two or three days and in all cases within the first week. The rapidity and extent of the rise was apparently somewhat proportional to the amount of lipocaic given. Usually the lipids rose to the preoperative level but occasionally a definite hyperlipemia was produced. This was however transitory and with continuation of adequate lipocaic therapy the lipid level remained at approximately the normal preoperative value. Coincident with the elevation of the depressed blood fats by lipocaic, the excretion of sugar increased, the insulin tolerance increased and liver function improved. The liver

fat although definitely decreased in two or three weeks usually did not return to normal until after five to six weeks of lipocaic therapy.

This effect of lipocaic in correcting the abnormal hypolipemia of depancreatized dogs has been employed by us as an additional criterion in the assay of various pancreatic extracts. It should be emphasized however that no one criterion is so reliable as the demonstration of a marked fatty infiltration of the livers in several depancreatized animals and their return to a normal state by the administration of the test substance.

CONCLUSION

1. The total serum lipids of insulin treated depancreatized dogs rise somewhat above normal during the first week after the operation and then gradually decline reaching a level approximately half of the normal in five or six weeks.

2. This hypolipemia is coincident with the development of marked fatty infiltration of the liver and the other signs of lipocaic deficiency.

3. The oral administration of lipocaic corrects this hypolipemia within a week and maintains the blood fat at the normal level.

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PANTING AND TEMPERATURE REGULATION IN THE CHICKEN

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Observations on panting were first reported by Richet (1898) who described two types which he designated as reflex-conditioned and central. Reflex panting was found to occur in non-anesthetized dogs even though no rise in body temperature took place. Central panting occurred in anesthetized dogs as a result of a rise in body temperature. Bazett's (1927) review of the literature concerning panting in cats and dogs includes the findings of Heymans and Heymans (1926) who used the isolated head preparation free from all afferent stimulation. In this preparation warming of the blood stream leading to the head produced increased respiration at 43° to 44°C. which was unquestionably of central origin. Central panting has been further investigated by Anrep and Hammouda (1932) who report that the temperature at which panting begins depends largely upon the depth of anesthesia. In light anesthesia panting may start at temperatures only slightly above normal, but if the anesthesia is deeper a considerable rise in body temperature must occur before panting starts. Very deep anesthesia was found to abolish panting altogether.

Hammouda (1933) studying the nervous control of panting found central panting to be due to a direct effect of heat on the optic thalami and reflex panting to be a conditioned reflex. Clark, Magoun and Ranson (1939) report that the size and location of lesions in the hypothalamus cause marked disturbances in temperature regulation of cats.

Von Saalfeld (1933) noted that certain reptiles when heated showed a "Warmepolypnoe" which served to cool the animal even though the mechanism involved is quite different from that causing panting in mammals.

Until 1936 very little attention had been directed toward temperature regulation of birds. Victorow (1909) mentioned that perhaps one of the functions of the air sacs might be protection from overheating of the body, but other than a few such observations little was known. In 1936 von Saalfeld, in a study of the respiration of doves, found a special ventilating mechanism which becomes active when the body is overheated, and which seems functionally similar to the panting mechanism in mammals. He

located a specific panting center in the anterior dorsal region of the midbrain which, when removed, leaves respiration solely under control of the respiratory center in the medulla. This panting center he found to be stimulated by a rise and depressed by a fall in temperature.

In reviewing the literature available, no data concerning such temperature regulation in the chicken have been found; it therefore is the purpose of this paper to present a study of the mechanism thus involved.

METHODS. A heating cabinet described elsewhere (Hiestand and Randall, 1939) was used for raising body temperature in adult barred-rock chickens. Respiratory movements were recorded by a pneumograph placed around the bird's thorax. Internal temperatures were recorded by a sensitive thermometer placed deeply in the cloaca. Unanesthetized birds were kept quiet by a web strap placed loosely around the wings. A specially designed animal board further prevented struggling. Since we wished to compare the efficiency of temperature regulation in the different birds each animal was subjected to identical conditions of increasing external (cabinet) temperatures. In general, respirations were recorded at every tenth degree change of body temperature, these being continued until a change of from one to four degrees had resulted.

In certain experiments after the maximal panting rate had been reached the chicken was left in the cabinet and the temperature slowly reduced to normal, while in others the hood of the cabinet was removed and a draft of cold air from a fan was directed upon the chicken. In either instance respirations were recorded at successive drops in temperature.

To determine the effects of centrally acting drugs on the panting mechanism both depressant and stimulant drugs were employed. Respiratory depression was produced by sodium pentobarbital (nembutal) intraperitoneally. For light narcosis 75 to 150 mgm. per kilo were given; for deep narcosis as much as 300 mgm. per kilo was used. To increase the activity of the respiratory center lobeline sulphate, 6.6 mgm. per bird, was injected subcutaneously. Paraldehyde, chloral hydrate, and tribromethanol (avertin) were also tried with some birds but no advantages over pentobarbital were noted. A total of 33 experiments was performed in this investigation.

RESULTS. *1. Panting and temperature regulation in the non-anesthetized chicken.* The normal body temperature was found to be subject to diurnal fluctuations but averaged about 42.5°C. As the external (cabinet) temperature was raised there occurred no acceleration of breathing until the body temperature had risen several tenths of a degree. In the majority of cases a fall of from 0.1° to 0.5° body temperature occurred soon after the bird was placed in a cabinet, the temperature not returning to "normal" until the environmental temperature had risen several degrees. With one notable exception in a total of 33 experiments, there was no acceleration

of breathing rate during this time. In this single instance the respiratory rate increased abruptly from 58 to 145 strokes per minute even though the body temperature had dropped 0.05°. During this time the temperature of the air in the cabinet had been raised from 23.5° to 37°.

With a rise of 0.1° to 0.4° in body temperature, the exact amount of the rise varying with different chickens, symptoms of panting began to appear. The beak was intermittently opened and closed, the head was raised, and often defecation with a considerable loss of water occurred. The graphs (fig. 1) show the effect of body temperature on panting rate in two birds having quite different responses to temperature change. Bird A is typical of most of the birds used showing a rather uniform and gradual increase in panting rate accompanying rising body temperature. Chicken B was a bird whose panting response was more abrupt as is shown by the sudden acceleration as panting began. Acceleration of this type indicates a more sensitive controlling mechanism than that of bird A and is not typical of most chickens. When the temperature of bird A was lowered slowly the return to the normal non-panting rate followed the ascending curve of increasing panting rate reasonably closely. This was true in every instance. However, if the bird was suddenly chilled by a blast of cold air, even though the air was directed only upon the head, reflex inhibition of panting resulted as shown by figure 1, B.

After respiration had reached a maximal rate at which it was maintained for a short time, a decided slowing occurred even though no reduction of or change in body temperature took place. This slowing of respiration invariably took place if the body temperature was maintained at a high point for a length of time.

With one chicken heating was continued for an exceptionally long time after the maximal panting rate was reached in order to determine the upper lethal temperature limit. In this bird the body temperature was raised to 47° and maintained for a brief time after which the bird died. A gradual decrease in panting rate occurred above 45.9° until death occurred.

In nearly every experiment the body temperature continued to rise after the external temperature had started to fall. In one instance even though the external temperature had dropped 7° in 10 minutes the internal temperature did not begin to fall until after this lapse of time.

2. *Panting and temperature regulation following the administration of central depressants.* In every instance where central depressants were administered respiratory depression occurred. The depth of the narcosis determined the degree of the depression. In lightly narcotized birds panting did not reach as rapid a rate as in the same birds non-anesthetized. In profound narcosis, panting was abolished altogether. Three noteworthy changes invariably occurred as a result of the action of central depressants: 1, the onset of panting was delayed, i.e., the panting temperature

threshold was raised; 2, the panting rate did not increase in proportion to body temperature as rapidly as in the same birds when not anesthetized, and 3, the maximal panting rate was less than in the non-anesthetized

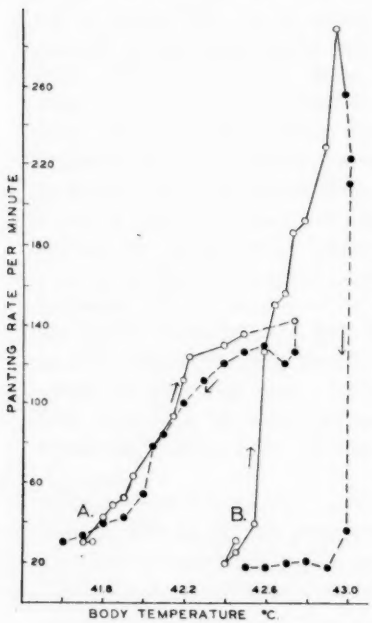

Fig. 1

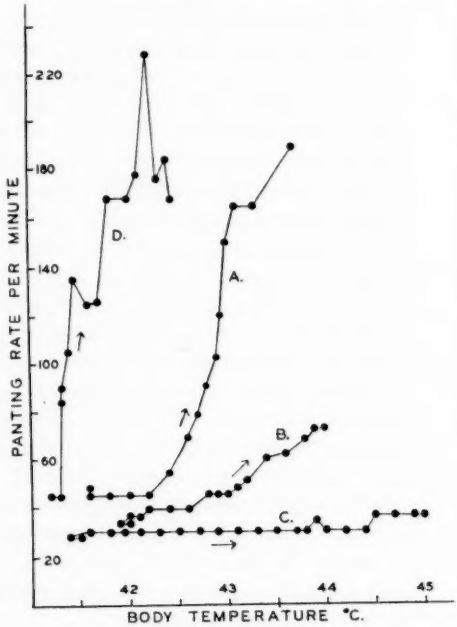

Fig. 2

Fig. 1. Panting rate in relation to body (cloacal) temperature in two chickens.

A. Chicken 21: The unbroken line indicates rising, dotted line falling, body temperatures.

B. Chicken 16: The unbroken line indicates rising body temperatures. Near the top of the graph (beginning of broken line) the hood of the cabinet was removed and a draft of cold air directed on the chicken. (Note cessation of panting about 2 minutes later.)

Fig. 2. A typical record of the effects of centrally acting drugs on temperature regulation in the chicken.

A. Normal record of panting rate in relation to body temperature (no drug used).

B. Same bird under light narcosis (nembutal, 150 mgm. intraperitoneally).

C. Same bird under deep narcosis (nembutal, 300 mgm. intraperitoneally).

D. Same bird after subcutaneous injection of 6.6 mgm. lobeline sulfate.

birds. Thus, when narcosis occurred, the maximal panting rate was never as great as in the non-anesthetized animal. Under light anesthesia, the birds responded slightly to afferent stimulation but none was disturbed by the procedure of the experiment. Under deep narcosis the birds could

not be aroused, nor did they exhibit any signs of reflex activity. Three of the birds died from overdose of the anesthetic. Whenever a depressant drug was used the comb became paler. The comb color was compared with a Tallquist hemoglobin scale and the color change recorded.

3. *Panting and temperature regulation following the administration of lobeline.* When injected subcutaneously, lobeline caused a prompt increase in breathing rate which was short lasting. To cite one instance: the breathing rate increased from a normal of 48 to 57 strokes per minute, two minutes after injection. A noticeable increase in amplitude also occurred. The respiratory stimulation was visibly evident for only about one and one-half minutes after the onset, however. In spite of the disappearance of external signs of stimulation, observations of panting later showed clearly that the lobeline effects persisted for at least the remainder of the experiment which occupied about one and one-half hours.

Each time lobeline was given and internal temperature raised, three effects were apparent. These were 1, an earlier onset of panting, viz., at a lower body temperature, indicating a lowered panting temperature threshold; 2, a greater increase in panting rate in relation to internal temperature, and 3, a greater maximal panting rate.

Discussion. That the ability to regulate body temperature by panting varies with different chickens is shown by these experiments. The panting temperature threshold also varies with different birds. The initial fall of body temperature from 0.1° to 0.5° after the warming has begun might be explained by reflex vasomotor changes as is known to occur in other homoiothermic animals (mammals). The continued rise in body temperature after the heat has been turned off is a phenomenon common to such experiments and dependent on the physical conditions determining the lag in changes in deep temperature when heat is absorbed through the surface.

After panting is inaugurated, acceleration is roughly proportional to body temperature. A true straight-line relationship does not occur in any instance, there being slight fluctuations on either side of the mean. The delay in acceleration following the increase in body temperature can be explained on the basis of subthreshold panting temperatures. As the rise in body temperature continues, a maximal point is ultimately reached at which the panting mechanism begins to fail as is apparent in a slowing of the rate. Failure may be due either to fatigue of the respiratory muscles or to heat damage to the central mechanism or to both.

With the one exception previously mentioned, no reflex acceleration of breathing occurred, the increase always lagging behind the rise in body temperature. The immediate slowing of the rate when the bird is placed in a draft of cold air shows definitely reflex inhibition of panting.

Von Saalfeld (1936) established the location of the panting center showing it to be separate from the respiratory center. That the panting center

is inactive at ordinary temperatures but has a certain critical temperature threshold is shown by these experiments. It can be assumed that the increase in breathing rate during panting is due to a master reaction response of the panting center when its threshold has been reached.

Central depressants apparently raise the threshold of the panting mechanism such that it may be said that the onset of panting is governed by two factors: 1, the internal temperature, and 2, the degree of excitability of the panting center. That central depressants affect the panting center before they do the respiratory center is evident from the fact that in deep narcosis respiratory movements continue at a regular rhythm independent of body temperature. In lighter narcosis the panting rate remains proportional to body temperature within limits, but at any given temperature above the panting threshold, the rate is less than for the unanesthetized bird at the same temperature.

Lobeline, a respiratory stimulant, produces opposite effects. The panting threshold is lowered by lobeline so that panting is produced with less rise of internal temperature. Likewise, it facilitates temperature control by producing a greater response at any given temperature above the normal threshold level. In short, the effects of lobeline may be explained on the basis of a sensitized panting center. The immediate and short-lived effects are those of direct stimulation probably of the respiratory center alone or of both centers. Small doses of lobeline are said to stimulate respiration by lowering the CO_2 threshold (Sollmann). This is indicated by the greater amplitude as well as increased rate of the respiratory movements. Von Saalfeld recorded the respiratory changes in doves after lobeline and noted an increase in breathing rate lasting several seconds, an increase in minute volume, but little change in depth of respiration.

The effect of lobeline on panting may be explained in two ways. It may lower the threshold of the panting center to heat as has already been postulated or it may increase the sensitivity of the respiratory center to the influence of the panting center thereby facilitating the response mechanism of the lower center. The first explanation seems the more plausible since we have seen that deep narcosis abolishes panting. However, it might be argued that narcosis raises the threshold of the respiratory center to a point where the latter is refractory to the discharges of the panting center. The end result of either explanation would be the same.

SUMMARY

1. Panting in the chicken begins at a certain critical internal temperature which varies with individual birds.

2. Respiratory acceleration (panting) is roughly proportional to body temperature within a certain temperature range.

3. When body temperature reaches a certain upper critical limit or when panting reaches a certain rate, the panting mechanism begins to fail.

4. Evidence of reflex production of panting was found in only one of thirty-three experiments.

5. Reflex inhibition of panting occurs if the chicken is suddenly chilled externally.

6. Central depressants depress panting depending upon the depth of the resultant narcosis.

7. Light anesthesia raises the panting temperature threshold; deep anesthesia abolishes panting entirely.

8. Evidence is shown that central depressants affect the panting center before the respiratory center itself is affected.

9. Respiratory stimulation by lobeline results in a direct and prompt but short lived effect manifest by an increase in the amplitude as well as rate of respiratory movements, followed by a longer lasting period of increased sensitization of the panting mechanism.

10. Lobeline lowers the panting heat threshold thereby increasing the efficiency of the temperature regulating mechanism of the chicken.

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PREPARATION AND BIOASSAY OF RENIN[1]

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Tigerstedt and Bergman (1) first called attention to a pressor substance, apparently protein in nature, extractable from the kidneys of various mammals. They named this blood-pressure-raising principle "renin." Later investigators have confirmed their observations in all essentials, and numerous attempts have been made to purify the pressor principle and to establish some of its chemical properties (2–10).

The work of Goldblatt (11–12) and others (13–23) on the production of experimental hypertension in dogs by means of renal ischemia has stimulated interest in renin as a possible causative agent for hypertension of renal origin.

Part 1 of the present communication is concerned with methods for extraction from hog kidneys, and purification of a non-toxic, powerful pressor protein apparently belonging to the group of globulins, and which we assume to be renin. Part 2 is a study of the bioassay of this substance.

I. *Extraction and purification of renin.* A fractional precipitation method, employing sodium chloride and ammonium sulphate was used, and the essential data are given in the flow sheet.

Fractionation Procedure for Preparation of Renin

Fresh hog kidney cortex—3 kgm. Extracted 24–48 hours at 6°C. with 9 liters 2 per cent NaCl sol. Strain through cheese cloth. Meat discarded. Liquid run through Sharples centrifuge. Tissue residue discarded.

Saline extract. To pH 4.5 using 10 per cent HCl. Allowed to stand 24 hours at 6°C. Ppt. removed with Sharples. Liquid filtered using Hyflo Super-Cel.[3]

Filtrate. To pH 6.8 using 10 per cent NaOH. Conc. to 1 liter in vacuo at 45°C.[4] Filtered with Hyflo.

Filtrate. NaCl concentration adjusted to 25 per cent. pH adjusted to 2.0–2.4 with 10 per cent HCl. Stored at 6°C. for 12 hours. Filtered with Hyflo.

Filter cake and ppt. Cake suspended in 5 liters N/5000 NaOH. Stored 12 hours at 6°C. after shaking for 40 minutes. pH to 6.5 with 10 per cent NaOH. Filtered.

[1] Aided by a grant from the Research Corporation, New York.

[2] E. R. Squibb & Sons Fellow in the Biological Sciences.

[3] A Celite Filter Aid, Johns-Manville Company, New York.

[4] The concentrated material may be stored without preservative for a period of at least 2 months if kept at 6°C.

Filtrate. Concentrated to 500 cc. in vacuo at 45°C. pH of the concentrate lowered to 2.0–2.4 using 10 per cent HCl. NaCl adjusted to 25 per cent. Allowed to stand 1–2 hours at 6°C. Centrifuged (International). Supernatant discarded.

Precipitate. Dissolved in 150–200 cc. water. pH to 4.0–4.5 using N/10 HCl. Filter and discard ppt. NaCl adjusted to 25 per cent and pH to 2.5 with N/10 HCl. Centrifuge after chilling at 6°C. Supernatant discarded.

Precipitate. Dissolved in 300–400 cc. M/10 acetate buffer, pH 5.00. Add solid $(NH_4)_2SO_4$ to 0.5 saturation. Suspension filtered using no. 512 Cel. Filter cake stored at 6°C. in small volume buffer as Filter cake 1.

Filtrate. Solid $(NH_4)_2SO_4$ to 0.7 saturation. Suspension filtered using no. 512 Cel. Filtrate discarded.

Filter cake 2. Ppt. dissolved from Cel with cold buffer sol. Cel filtered off. Filtrate to 0.5 saturation with $(NH_4)_2SO_4$. Suspension filtered with no. 512 Cel. Filtrate discarded.

Filter cake 3. Combined with filter cake no. 1. Ppts. dissolved from Cel with cold buffer sol. Cel filtered off. Filtrate to 0.4 saturation with $(NH_4)_2SO_4$. Suspension filtered with no. 512 Cel. Filter cake 4.

Filtrate: 0.5 saturation with $(NH_4)_2SO_4$. Suspension filtered using no. 512 Cel. Filter cake 5.

Filtrate: to 0.6 saturation with $(NH_4)_2SO_4$. Suspension filtered with no. 512 Cel. Filtrate discarded.

Filter cake 6. Combined with filter cake 4. Ppts. dissolved from Cel with buffer. Cel filtered off. Filtrate to 0.5 saturation with $(NH_4)_2SO_4$. Suspension filtered with Cel. Filtrate discarded.

Filter cake 7. Combined with filter cake 5. Ppts. dissolved from Cel with buffer. Fractionation between 0.4, 0.5 and 0.6 saturated $(NH_4)_2SO_4$ repeated. Final 0.5 saturated fraction dissolved in 40–50 cc. distilled water and dialyzed at 6°C. for 24 hours.

Final extract: 4–6 mgm. solids/cc. Total nitrogen content 15.4–15.7 per cent of the total solids. Non-toxic.

The active pressor substance is positive for all of the standard protein tests, and insofar as its behavior to the salting out procedure is concerned, it is apparently a globulin.

The extract will retain its activity for a long period if kept solidly frozen. If lyophiled (27) and kept in the dry state as a powder under vacuum, it will retain its activity for two months and probably longer, although it has not been tested beyond 60 days. The fact that it was such a simple matter to keep the material led us to undertake the bioassay of renin.

II. *Bioassay of renin.* Few efforts to assay relatively pure, stable preparations of renin have been made. Most investigators have confined their attention to extraction and purification studies or to the physiological action of the preparations. Others have compared the pressor effect of extracts of normal kidneys with extracts of ischemic kidneys of the same species.

Pickering and Prinzmetal (7) prepared various types of renin extracts and tested their effects on the blood pressure of unanesthetized rabbits by the "ear capsule" method. They made assays of renin and state that the

relationship of the amount of renin administered, to the blood pressure rise obtained, is not linear but is approximately so on a logarithmic scale. A standard was set up for comparative purposes and, on the average, this standard gave a rise of 20 to 30 mm. Hg with 0.25 cc. renin extract for the adult rabbit.

Helmer and Page (10), in their study on the purification and properties of renin, state that the cat is a favorable form for assay purposes, and that assays will be discussed in detail in a forthcoming communication. Using the cat, their purest preparation of renin, containing 0.009 mgm. of nitrogen, produced a rise in arterial pressure of 32 mm. Hg.

MATERIALS AND METHODS. For purposes of bioassay we have employed the rise in blood pressure exhibited by normal, anesthetized dogs after intravenous injections of known amounts of stable renin preparations. The dosage levels were calculated on the basis of the solid content of the extract and the weight of the animals. A single preparation of renin prepared according to the method described was used in all cases reported here. Other batches of renin prepared by this procedure gave comparable results.

The dogs were anesthetized with pentobarbital sodium (Nembutal), representing 0.6 cc. per kgm. body weight. All renin injections were made through the jugular vein, total volume of fluid representing 0.07 cc. per kgm. body weight.

Blood pressure was determined in the femoral artery by the needle puncture method (24, 25) and recorded by means of a Tycos sphygmomanometer. Arterial pressures as determined by this method are mean pressures. Continuous readings can be taken for 10 to 15 minutes without clots forming, if an anticoagulant is used in the needle. In all of the assay work, two complete blood pressure outfits were used. When clots formed in the needle of one, the other apparatus was used on the femoral artery of the other leg. It was thus a simple matter to secure continuous readings over long intervals. The usual method was to record the pressure readings every 15 seconds for the first 10 minutes and then every minute for the next 10 minutes. Following this interval the pressures were recorded every 5 to 10 minutes.

Following an injection of renin, the peak rise in pressure is reached within the first 3 to 4 minutes, on the average, after which the fall to normal is slow and may be prolonged over an hour. It has been our experience that after the dog has been under pentobarbital sodium for two hours the pressure readings are not reliable because the animal has begun, by this time, to recover from the anesthesia. Except in special experiments each assay dog received but one renin injection every 72 hours.

The blood pressure of normal dogs varies greatly among individuals, and as recorded by the needle puncture method, the pressure of different dogs

may vary from 95 to 150 mm. Hg (25). However, the arterial pressure of individual animals maintained under uniform conditions varies surprisingly little. Among the large number of animals studied by us during the past few years, we have recorded individuals which we designate as "high pressure" dogs, i.e., individuals in which the normal mean arterial pressure registered 140 to 150 mm. Hg under pentobarbital sodium anesthesia, no matter how many times they were anesthetized. Numerous "low pressure" animals have also been observed, in which the mean pressure under pentobarbital sodium varied from 90 to 98 mm. Hg.

TABLE 1

Renin injections representing 0.1 mgm. solids (0.016 mgm. nitrogen) per kilogram body weight

DOG NUMBER	WEIGHT	INITIAL B.P.	PEAK B.P.	NUMBER MM. RISE B.P.	TIME TO PEAK		REMARKS
Injections made over a period of 2 to 3 seconds							
	kgm.	*mm. Hg*	*mm. Hg*		*min.*	*sec.*	
1	11.5	109	159	50	3	0	B.P. 135 at 34 min., 132 at 64 min.
2	8.2	141	181	40	3	30	B.P. 179–181 for 2 min.
3	11.9	132	172	40	4	0	B.P. 155 at 24 min.
4	12.1	113	145	32	2	45	B.P. back to 112 in 19 min.
5	15.3	137	170	33	3	0	B.P. 149 at 13 min.
6	14.1	127	168	41	2	45	B.P. 142 at 19 min., 122 at 60 min.
7	8.9	122	159	37	4	0	B.P. 140 at 16 min., 117 at 34 min.
8	12.1	110	147	37	4	0	B.P. still 147 at 7 min.
9	8.3	114	166	52	3	30	B.P. 140 at 9 min.
Unanesthetized animal							
10	11.1	130	178	48	2	0	B.P. 157 at 8 min.
10	11.1	128	170	42	2	0	
10	11.1	136	178	42	2	0	B.P. 170 at 5 min.
Averages...		124.9	166.1	41.2	3.0		

The renin used for assay was lyophiled and kept as a powder under vacuum until ready for use. The renin solution used for injections contained 7 mgm. of solids per cubic centimeter and the total nitrogen content was 15.4 to 15.7 per cent of the total solids. The solid content of this preparation of renin was somewhat greater than most of our material.

Assay of renin. The essential data obtained from study of the blood pressure change following injections of 0.1 mgm. of solids per kgm. body weight are given in table 1. The tabulated results reveal several interesting facts.

1. The pentobarbital sodium employed for anesthesia apparently does not alter the blood pressure response. As a check on this point, trained, unanesthetized dogs were used. The pressure responses of such animals do not differ significantly from those of the anesthetized animal.

2. The arterial pressure rise attains its peak at approximately 3 minutes after injection and slowly declines to the starting level of pressure. Practically all investigators who have studied the pressor effects of renin extracts have commented upon the prolonged action of the material.

3. Renin injections representing 0.1 mgm. solids and 0.016 mgm. nitrogen, when given intravenously over a period of 2 to 3 seconds, raise the mean arterial pressure an average of 41.2 mm. Hg over the initial starting level of pressure of the anesthetized or unanesthetized dog.

4. It was observed that at the 0.1 mgm. solids per kgm. dosage, the initial or starting pressure level at the time of injection apparently did not interfere with the blood pressure response to renin. As will be shown later, when larger doses of renin are used, the degree of arterial pressure response will depend partially, in most cases, upon the initial or starting pressure level.

Because the responses to small doses of renin are apparently not affected by the initial or starting level of pressure, we selected the average arterial pressure rise of at least 40 mm. Hg, in response to 0.1 mgm. solids per kgm. body weight, as the standard with which to compare the effectiveness of all higher dosage levels.

Renin injections representing 0.2 mgm. solids per kgm. body weight (*table 2*). Twenty-one dogs were used for assay at this dosage level. In 5 the injections were given over a 2 to 5 second interval, in 12 others the injections lasted 30 seconds, and in 4, an injection period of 60 seconds was used. No significant difference in response was observed between the groups receiving injections over 2 to 5 and 30 seconds. The average mean blood pressure rise for the 17 dogs in these two groups was 71.9 mm. Hg above the starting level. On the other hand, the 4 dogs in which the injection interval was 60 seconds gave less marked pressure increases.

It was mentioned earlier that the initial level of blood pressure of the test animal previous to injection apparently has an important bearing upon the degree of responsiveness to renin at certain dosage levels. In general it can be said that if the pressure is high at the time of injection the response will be less than if the starting pressure were lower. Although this statement seems not to apply to doses of 0.1 mgm. solids per kgm. or lower, it generally holds true for the higher doses. Table 3 illustrates the dampening effect of relatively high initial mean arterial pressures upon the response to renin. The average rise in pressure of 4 such dogs was but 43.5 mm. Hg when the injections were administered over a 30 second interval, and but 31.5 mm. Hg when the rate of injection was slowed to 1

minute. As shown in table 2, however, the average mean pressure rise for the same dose in dogs with lower starting pressures was 71.9 mm. Hg (compare tables 2 and 3).

TABLE 2

Renin injections representing 0.2 mgm. (0.032 mgm. nitrogen) per kilogram body weight

DOG NUMBER	WEIGHT	INITIAL B.P.	PEAK B.P.	NUMBER MM. RISE B.P.	TIME TO PEAK		REMARKS
	kgm.	*mm. Hg*	*mm. Hg*		*min.*	*sec.*	
Injections made over a period of 2 to 5 seconds							
1	11.5	126	191	65	5	30	B.P. 137 at 51 min.
2	11.5	94	158	64	3	15	B.P. 118 at 35 min., 101 at 70 min.
3	10.4	93	148	55	6	0	
4	14.7	114	191	77	4	45	B.P. 154 at 42 min.
5	10.1	101	194	93	3	45	B.P. 140 at 36 min., 130 at 60 min.
Injections made over a period of 30 seconds							
6	9.8	101	156	55	3	15	B.P. 142 at 6 min.
7	11.9	118	174	56	3	30	B.P. 138 at 30 min., 137 at 50 min.
8	14.1	107	182	75	2	45	B.P. 143 at 31 min.
9	11.3	105	185	80	3	45	B.P. 180–185 for 4 min.
10	11.7	92	184	92	3	0	B.P. 115 at 40 min.
11	14.3	108	204	96	4	0	B.P. above 200 for 2 min.
12	10.0	107	163	56	3	15	B.P. remained at peak 3 min.
13	10.2	116	188	72	3	15	B.P. 160 at 8 min.
14	13.0	102	181	79	4	30	
15	9.8	116	173	57	4	0	B.P. 135 at 10 min.
16	8.6	113	172	59	3	45	B.P. 151 at 10 min.
17	10.9	115	207	92	4	30	
Averages...		107.5	179.4	71.9	3.8		
Injections made over a period of 60 seconds							
18	13.6	129	190	61	4	0	B.P. 172 at 10 min.
19	11.9	98	149	51	4	0	
20	14.1	124	171	47	4	0	B.P. 162 at 9 min.
21	10.0	101	162(?)	61			Missed the peak
Average.....				55			

Renin injections representing 0.3 mgm. solids per kgm. body weight (table 4). Seventeen dogs were used for tests of renin at this dosage level. The injection time was 30 seconds for 6 animals and 60 seconds for 6 others. Since no observable difference in response was noted, they are considered as one group in the table. The average mean pressure rise for the group

TABLE 3

Renin injections representing 0.2 mgm. solids per kilogram body weight, using dogs with relatively high pressures

DOG NUMBER	WEIGHT	INITIAL B.P.	PEAK B.P.	NUMBER MM. RISE B.P.	TIME TO PEAK		INJECT. TIME	REMARKS
	kgm.	*mm. Hg*	*mm. Hg*		*min.*	*sec.*	*sec.*	
1	9.1	138	179	41	2	45	30	B.P. 153 at 17 min.
2	13.8	147	187	40	4	0	30	B.P. 148 at 23 min.
3	11.5	140	186	46	2	0	30	B.P. 182 at 8 min.
4	8.1	138	185	47	3	0	30	
Average				43.5				
5	12.4	131	174	43	5	0	60	
6		140	160	20	4	0	60	
Average				31.5				

TABLE 4

Renin injections representing 0.3 mgm. solids (0.048 mgm. nitrogen) per kilogram body weight

DOG NUMBER	WEIGHT	INITIAL B.P.	PEAK B.P.	NUMBER MM. RISE B.P.	TIME TO PEAK		INJECT. TIME	REMARKS
	kgm.	*mm. Hg*	*mm. Hg*		*min.*	*sec.*	*sec.*	
1	11.7	118	178	60	3	45	2–5	B.P. 148 at 52 min.
2	10.5	118	178	60	4	30	2–5	B.P. 118 at 68 min.
3	12.3	116	182	66	4	15	2–5	
4	11.8	112	180	68	5	0	2–5	
5	13.5	116	176	60	6	0	2–5	
Average				62.8				
6	12.6	99	212	111	4	30	30	B.P. 158 at 45 min.
7	14.5	110	219	109	3	30	30	B.P. 148 at 60 min.
8	10.2	116	211	95	3	30	30	B.P. 120 at 31 min.
9	10.1	114	195	81	3	0	30	B.P. 144 at 56 min.
10	14.3	108	195	87	4	45	30	B.P. 160 at 22 min.
11	10.2	119	205	86	3	30	30	B.P. 152 at 30 min.
12	8.7	98	214	116	3	30	60	B.P. 178 at 8 min.
13	10.2	103	209	106	4	15	60	B.P. 158 at 10 min.
14	10.5	114	233	119	3	45	60	B.P. 157 at 10 min.
15	11.8	97	192	95	4	0	60	B.P. 166 at 20 min.
16	11.2	119	214	95	3	15	60	B.P. 192 at 10 min.
17	11.7	130	192	62	3	45	60	B.P. 190 at 6 min.
Averages...		110.6	207.6	97.0	3.8			

was 97 mm. Hg above the starting level. The average peak pressure attained was 207.6 mm. Hg. Only 1 dog (no. 17, table 4) had a high starting pressure and this animal exhibited the least response.

Effect of rate of injection upon blood pressure response at the 0.3 mgm. dosage level (table 4). A group of 5 dogs was injected with the same dose of renin (0.3 mgm. solids per kgm.) as the remainder of the animals tested in table 4, but the injection time was reduced to 2 to 5 seconds. The pressure response to such rapid injections was less than when a similar injection was given over a 30 to 60 second interval. The average rise for the quick injections was 62.8 mm. Hg whereas it was 97 mm. Hg above the starting level in the group receiving slower injections (table 4).

TABLE 5

Renin injections representing 0.4 mgm. solids (0.064 mgm. nitrogen) per kilogram body weight

All injections made over a period of 60 seconds

DOG NUMBER	WEIGHT	INITIAL B.P.	PEAK B.P.	NUMBER MM. RISE B.P.	TIME TO PEAK		REMARKS
	kgm.	*mm. Hg*	*mm. Hg*		*min.*	*sec.*	
1	10.4	98	224	126	2	45	B.P. 145 at 14 min.
2	12.4	105	200	95	3	30	B.P. 179 at 6 min.
3	10.3	84	200	116	3	45	B.P. 131 at 20 min.
4	12.0	100	218	118	3	0	B.P. 147 at 23 min.
5	11.2	116	230	114	2	45	B.P. 160 at 30 min.
6	10.3	117	226	109	3	0	B.P. 138 at 24 min.
7	11.9	96	191	95	3	30	B.P. 154 at 10 min.
8	14.0	93	209	116	3	45	B.P. 142 at 31 min.
9	13.5	120	230	110	4	0	B.P. 165 at 30 min.
3	10.1	94	226	132	3	45	B.P. 165 at 11 min.
10	12.6	128	236	108	4	0	B.P. 173 at 25 min.
11	13.5	94	195	101	4	30	B.P. 145 at 30 min.
Averages....		103.8	215.4	111.7	3.5		

Renin injections representing 0.4 mgm. solids per kgm. body weight (table 5). Twelve dogs were employed. Six could be classed as initial "low pressure" dogs, and 1 (no. 3) is recorded as having a pressure of 84 mm. Hg. This is probably an erroneous reading, since the same animal, when used a few days later, is recorded as having an initial mean pressure of 94 mm. Hg (table 5). The average rise in blood pressure at 0.4 mgm. dose level was 111.7 mm. Hg above the initial pressure. The average peak pressure for the group was 215.4 mm. Hg. The optimum injection time for this dosage level was 1 minute.

Since the highest mean pressure response we have obtained with our renin preparations was 256 mm. Hg in a dog with a starting pressure of

101 mm. Hg, we made no attempt to assay at a higher dosage level than 0.4 mgm. solids per kgm. body weight. Several of the peak pressures recorded in table 5 closely approach the maximum we have ever observed with any dose (table 6). Hence it seemed improbable that higher doses of our preparation, e.g., 0.5 mgm. solids per kgm., would have given much greater responses than those obtained at the 0.4 mgm. dose level. Injecting 8 to 9 times this amount of renin, e.g., 35 mgm., failed to evoke greater blood pressure rises (table 6).

The relationships between renin dosage and average blood pressure response is shown graphically in figure 1. The response is directly proportional to dosage for all levels tested except 0.4 mgm. However, the blood pressure response to this dose level over the 0.3 mgm. level is statistically significant.

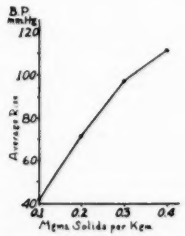

Fig. 1. Relationship between renin dosage and average blood pressure response; 0.1 mgm. per kgm. body weight tested in 12 animals; 0.2 mgm. tested in 17 animals; 0.3 tested in 17 animals; 0.4 mgm. tested in 12 animals.

For convenience in assaying the potency of fractionation products, we have adopted as a unit *the minimum amount of renin necessary, when given intravenously over a period of 2 to 5 seconds, to raise the mean arterial pressure of anesthetized dogs an average of 40 mm. Hg above the starting pressure levels.* Not less than 10 injections, preferable to different animals, should be used to establish the average. This unit involves a range of pressure rise which is not influenced by individual variations in the normal starting pressure. Insofar as our preparation is concerned, the unit is equivalent to 0.1 mgm. solid material per kgm. body weight and contains 0.016 mgm. nitrogen.

Effect of repeated renin injections on blood pressure response of the unanesthetized dog. If repeated injections of renin are given at short intervals, the blood pressure response is diminished to all injections after the first one (2, 7, 8, 15, 22). We have repeatedly observed this lessening of response to equivalent doses when they are given in rapid succession. It was pointed out by Bingel and Strauss (2) and Pickering and Prinzmetal (7), however, that no diminution of response occurs if each successive injection is withheld until the blood pressure effect of the previous injections

has disappeared. We can confirm these observations from studies on the trained unanesthetized dog. No diminution in the pressor response to repeated renin injections occurs if the injections are so timed that all pressor effects of previous injections have disappeared before the subsequent ones are administered.

The question of toxicity of renin preparations. During the course of the experiments on extraction, purification and assay of renin, we have employed 107 dogs and have given more than 500 intravenous injections of extracts in various stages of purification. We have observed toxic responses in 5 animals, 4 of which died within 12 hours following injection, and 1 recovered but exhibited paralysis of the hind legs. These 5 dogs received large intravenous doses (5–10 cc.) of crude extracts and in all cases the blood pressure response was marked. Since the dogs had previously

TABLE 6

Effect of large injections of renin on dogs previously injected with smaller doses

DOG NUMBER	NUMBER PREVIOUS INJECTIONS	INTERVAL BETWEEN INJECTIONS*	INITIAL B.P.	PEAK B.P.	NUMBER MM. RISE B.P.	NUMBER MGM. INJECTED	REMARKS
		days	*mm. Hg*	*mm. Hg*			
1	7	8	118	227	109	35	No signs of toxicity
2	6	17	102	207	105	35	No signs of toxicity
3	7	8	106	218	112	35	No signs of toxicity

* The number of days between the last small injection and the 35 mgm. dose.

received several small injections, we suspected anaphylaxis. However, with further fractionation and purification of the material, we failed to obtain any evidence of toxicity or anaphylactic phenomena even when massive doses of renin were given (table 6).

Previous workers who have experimented with renin extract agree that they are non-toxic (7, 9).

SUMMARY

1. A fractional precipitation method, employing sodium chloride and ammonium sulfate, for the extraction and purification of renin, is described. Organic solvents have not been employed in this procedure.

2. The final product obtained is a non-toxic pressor protein apparently belonging to the globulin group.

3. The protein retains its pressor properties unimpaired for long periods if kept solidly frozen, or if lyophiled and kept in the dry state as a powder under vacuum.

4. The frozen or dried material contains 4 to 6 mgm. of solids per cubic centimeter. The total nitrogen content is 15.4 to 15.7 per cent of the total solids.

5. The pressor principle is non-dialyzable and is positive for all of the standard protein tests.

6. A relatively pure and stable preparation of renin was assayed at various dosage levels (0.1, 0.2, 0.3, and 0.4 mgm. solids per kgm. body weight) on dogs, and a unit established.

7. A renin unit is defined as the minimum amount necessary, when given intravenously over a period of 2 to 5 seconds, to raise the mean arterial pressure of anesthetized dogs an average of 40 mm. Hg above the starting pressure level.

8. This unit in terms of the preparation used, is equivalent to 0.1 mgm. solid material per kgm. body weight and contained 0.016 mgm. nitrogen.

9. The blood pressure response to renin injections is directly proportional to dosage for all levels tested, up to and including the 0.3 mgm. level. Higher doses do not give proportional pressor responses.

10. Various factors which modify the pressor response, such as starting level of pressure, rate of injection, etc., are discussed.

11. The renin preparation employed was non-toxic even when intravenously injected in large (35 mgm.) amounts.

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THE EFFECT OF CORTIN AND DESOXYCORTICOSTERONE ACETATE ON THE ABILITY OF THE ADRENALECTOMIZED RAT TO INACTIVATE HISTAMINE

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In a previous communication it has been shown that the destruction or inactivation of intravenously injected histamine in the rat takes place mostly in tissues which do not contain histaminase (Rose and Browne, 1938). It was further observed that whereas the distribution of intravenously injected histamine is not altered following adrenalectomy, there is a marked decrease in the ability of various tissues to inactivate the amine.

Since it has been shown by previous workers that the resistance of the adrenalectomized rat to histamine may be restored to normal by the administration of adreno-cortical hormone (Gottesman and Perla, 1931) it was decided to study the effect of the administration of cortin and of desoxycorticosterone acetate on the ability of the adrenalectomized rat to inactivate histamine. A preliminary report of this work has been given (Rose and Karady, 1939).

METHODS. Since these experiments were carried out as a continuation of those previously reported, similar methods were used and identical conditions were maintained. Rats of a hooded strain weighing between 180 and 220 grams were used. The animals were adrenalectomized, placed on a diet of purina chow and given 0.85 per cent saline to drink. Seven days after the adrenalectomy was performed, at which time the decrease in resistance to histamine is well established, treatment with either cortin (Adrenal Cortex Extract, Wilson) or desoxycorticosterone acetate was started and maintained for four days.

Twelve hours after the last injection of either cortin or desoxycorticosterone acetate, the animals were anesthetized and a dose of histamine was injected intravenously. The salt used was histamine dihydrochloride dissolved in normal saline and the volume of fluid injected never exceeded 2 cc. The dose of histamine was administered according to the weight of the animal, namely, 24 γ as base per gram (or 0.04 mgm./gm. of the

[1] Aided by a grant from the Banting Research Foundation, Toronto, Canada.

hydrochloride). All histamine values are expressed as base unless otherwise indicated.

The animals were allowed to recover from the anesthesia and after a period of one, two or three hours, they were again anesthetized, the abdomen opened and a sample of blood removed from the inferior vena cava. The kidneys and liver were also removed. Six animals were used in each group, two animals being killed at the first, two at the second and two at the third hour after the injection of histamine. Thirty-six animals were treated in all.

The blood histamine was determined by the method of Barsoum and Gaddum (1935) as modified by Code (1937). Tissue histamine was determined by the method of Best and McHenry (1930). All assays were carried out on the isolated guinea-pig-ileum preparation suspended in tyrode solution to which was added atropine in a concentration of 1×10^{-7}.

TABLE 1

The rate of disappearance of intravenously injected histamine from the blood and tissues of the normal rat as compared with that in the adrenalectomized animal

DATA	HISTAMINE BLOOD, γ/cc.			HISTAMINE LIVER, γ/GRAM			HISTAMINE KIDNEY, γ/GRAM		
	1st hour	2nd hour	3rd hour	1st hour	2nd hour	3rd hour	1st hour	2nd hour	3rd hour
Normal animals	3.0	0.5	0.2	8.0	4.0	4.0	188	17	4.0
Adrenalectomized animals	12.0	10.0	7.0	53	25	7.0	388	290	95

RESULTS. In the previous experiments (Rose and Browne, 1938) it was established that if a dose of 24 γ of histamine per gram body weight were injected intravenously into a rat, the histamine disappeared from the blood and tissues at a definite rate. It was furthermore observed that the difference in the ability of the tissues of the normal animal to inactivate histamine as compared with those of the adrenalectomized animal was well marked at the first and second hours.

Table 1 shows the values obtained in blood, liver and kidney tissue following the intravenous injection of histamine in the intact animal as compared with those obtained in the adrenalectomized animal maintained on 0.85 per cent saline without any treatment. Each figure is the average of six different results. It will be observed that the histamine disappears much more slowly in the adrenalectomized animal.

Effect of cortin. In the first group of experiments the preparation used was Adrenal Cortex Extract (Wilson)[2] of which 0.5 cc. daily injected

[2] Supplied through the courtesy of Dr. David Klein and the Wilson Laboratories, Chicago, Ill.

intramuscularly is regarded as a maintenance dose for the rat. Two groups of six animals each were therefore treated with 0.5 cc. daily for four days. Twelve hours after the last dose they were given an intravenous injection of histamine and the blood and tissues removed as described above. The results may be seen in table 2. It will be seen that there was little or no change in the rate of disappearance of histamine as compared with that in the untreated adrenalectomized animals.

Two more groups were treated with larger doses of cortin and it will be observed that when cortin was given in a dose of 2 cc. twice daily for three days followed by 2 cc. four times on the fourth day, the rate of disappearance of histamine from blood and tissues was comparable to that in the intact animal.

TABLE 2

Effect of varying doses of cortin and of desoxycorticosterone acetate on the rate of disappearance of intravenously injected histamine in adrenalectomized animals

DATA	HISTAMINE BLOOD, γ/CC.			HISTAMINE LIVER, γ/GRAM			HISTAMINE KIDNEY, γ/GRAM		
	1st hour	2nd hour	3rd hour	1st hour	2nd hour	3rd hour	1st hour	2nd hour	3rd hour
Adrenalectomized animals, cortin 0.5 cc. daily for 4 days	11.5	8.8	2.9	34.0	14.7	9.9	224	206	81.0
Adrenalectomized animals, cortin 2 cc. daily for 2 days and 2 cc. × 2 for 2 days	8.4	3.5	1.7	25.7	6.4	3.3	265	124	37.7
Adrenalectomized animals, cortin 2 cc. × 2 for 3 days and 2 cc. × 4 for 1 day	1.8	1.0	0.45	15.1	3.9	2.5	81	13.8	9.7
Adrenalectomized animals, desoxycorticosterone acetate 2.5 mgm. × 2 for 3 days and 5.0 mgm. × 2 for 1 day	6.5	3.2	0.7	20.0	6.4	3.1	236	53	11.0

Effect of desoxycorticosterone acetate. In a second series of experiments, two groups of six animals which had been adrenalectomized and maintained on 0.85 per cent saline were given desoxycorticosterone acetate[3] in oil for four days. The preparation used contained 5 mgm. of the acetate per cubic centimeter. Each animal received 2.5 mgm. injected intramuscularly twice daily for 3 days followed by 5.0 mgm. twice daily on the fourth day. They then received the intravenous injection of histamine twelve hours after the last injection of the desoxycorticosterone acetate. The results are given in table 2. It will be noted that the rate of disappearance of histamine from the blood and tissues is comparable to that which took

[3] Supplied through the courtesy of the Ciba Company, Montreal, Canada.

place in the group of animals which received 2 cc. of cortin daily for two days followed by 2 cc. twice daily for two days.

Discussion. It will be noted that in comparison to the maintenance dose of cortin, a relatively much larger dose is necessary to restore the ability of the organism approximately to normal as judged by the rate of histamine inactivation. Similarly, a very large dose of desoxycorticosterone acetate is necessary to restore the ability of the animal organism to destroy histamine. The work of Kendall (1938) is interesting in this respect. He showed that whereas desoxycorticosterone is fifteen to twenty times as active as corticosterone, the amorphorus portion of cortical extracts is forty times as active, based on the daily amount required to maintain the blood urea of adrenalectomized dogs between 45 and 55 mgm. per cent. Waterman et al. (1939) on the other hand found that 1 mgm. of desoxycorticosterone acetate had the same effect as 20 to 30 cc. of cortin in the dog.

The inactivation of histamine in the animal organism may take place by several different mechanisms. Of these histaminase is the best understood (Best and McHenry, 1930). In other experiments it has been shown that the only organs of the rat which contain histaminase are lung and small intestine (Rose, Karady and Browne, 1939). Since destruction of intravenously injected histamine in the rat occurs mainly in those tissues which do not contain histaminase it would seem therefore that in this species, at least, some other means of histamine destruction must be responsible for the greater part of the histamine destroyed. It has been shown that following adrenalectomy, the histaminase content of the lung of the rat is diminished, and that this diminished histaminase content may be restored to approximately normal levels by the administration of cortin to adrenalectomized animals (Karady, Rose and Browne, 1939). In view of the above discussion however it does not seem probable that this change is the main factor in the alterations in the rate of histamine destruction following adrenalectomy and treatment with cortin.

From the above results it would seem that the adrenal medulla plays little or no rôle in the ability of the rats to inactivate histamine since animals treated with cortin alone are able to inactivate the amine as rapidly as the intact animal.

SUMMARY

1. The ability of the adrenalectomized rat maintained on 0.85 per cent saline to inactivate histamine may be restored by the administration of adrenal cortex extract or of desoxycorticosterone acetate.

2. The maintenance dose of cortin for a rat is wholly inadequate to restore the ability of the animal to inactivate histamine.

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ANALYSIS OF THE EMPTYING OF SEGMENTS OF THE ARTERIAL RESERVOIR[1]

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In a recent study of the pulse in the aorta (6) we had occasion to produce a great many aortic occlusions. The necessary confirmation of the effectiveness of occlusion and release furnished a large number of records of pressure fall at a point beyond the occlusion. An example was shown in the previous paper, and it was pointed out that an analysis of such curves should shed light on the relationships between elasticity and outflow in the arterial tree. The present paper describes the analysis and presents the results of a fairly extensive and representative series.

METHOD. All the experiments reported were done on dogs under ether after premedication with morphine sulfate 10 mgm. per kgm. The chests were not opened and artificial respiration was not necessary.

Internal occlusion of the aorta was accomplished by the inflation of a thin rubber balloon at the tip of a long cannula run up from the right femoral artery. The curves of fall of pressure in the distal segment were recorded through a small glass cannula tied into an artery near the left knee.

All pressures were recorded optically, by Hamilton metal-membrane manometers, on 6 cm. paper at a speed of about 4 cm. per sec. The records were measured by the method previously described (6), using an accurate 0.1 mm. measuring plate and low-power binocular magnification. The measurements were converted into pressures, as usual, by reference to smoothed curves of calibration of the membranes against a mercury manometer. The measured pressures may all be too high or too low by as much as 3 or 4 mm. Hg. However, with the exception of occasional minor difficulties mentioned later, the methods of calibration, measurement, and conversion should make the points on any one curve consistent with each other within 1 mm. Hg.

Most of the effective occlusions lasted from 4 to 10 sec. (one 15 sec. and

[1] Reported before the American Physiological Society at the meetings in Toronto, 28 April, 1939.

[2] On leave of absence, 1939–40, in the Department of Physiology, Western Reserve University School of Medicine, Cleveland.

one 24 sec.). Because of the usual interference of diastolic standing waves, readings were started wherever the curve appeared to have begun its smooth sag. This was nearly always at a pressure between 80 and 100 mm. Hg. When small pulsations occurred before occlusion became complete they were eliminated by closing up the time scale to the point where the falling pressure again reached the value it had had at the beginning of the pulsation. If these interruptions persisted far down the curve, indicating a continued supply of blood to the segment, the records were discarded.

RESULTS. Figure 1 illustrates the type of curve considered. The two upper lines show the pressure waves in the aorta above the occlusion and were the subject of discussion in the previous paper. The lower curve, *K*, is the one which is analyzed here. Four dogs furnished the thirty-five curves selected for measurement. Those obtained from one animal are presented in detail; the others yielded closely parallel results.

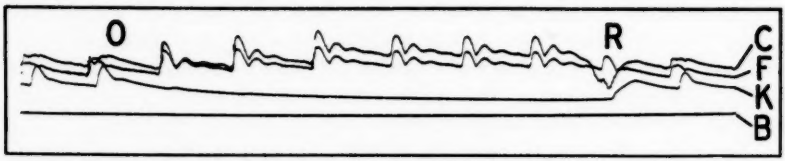

Fig. 1. Type of record analyzed. *C*, cannula down from carotid; *F*, cannula up from right femoral; *K*, cannula at left knee (the record here analyzed); *B*, common base line. The occluding balloon was at the tip of cannula *F*. Records *F* and *C* are from the aortic segment above the occlusion, and in this analysis are extraneous. Occlusion was begun at *O* and released at *R*. Time intervals 1/12 sec.

Figure 2 shows reconstructions of several records of pressure fall in one dog. The two lowest curves represent the extremes of variation in eight occlusions 27 cm. up from the femoral entrance—the other six lay between these two. The two upper curves are the result of occluding at 20 and 15 cm. respectively (each is one of three or four closely checking records).

One noteworthy feature of the results is evident upon inspection of this figure: the large number of closely spaced measurements permits the drawing of a smooth curve with little hesitation about the course it should take, and makes it easy to distinguish between accidental jogs and general trends. Most of the minor deviations occurred when a small early pulsation was eliminated or when the measuring plate (whose total width spread over about 1 sec. on the record) was shifted and re-aligned with the base line. One deviation occurred with sufficient regularity to justify its indication by the divergence of the solid and dotted ends of the lower curves. After the curves had become nearly level, usually about 8 sec. after occlusion, they might drop with comparative abruptness to level off about 2 mm. Hg below. One branch was therefore extrapolated from the

higher points while the other was plotted with nearly the same curvature to end in the lower points. The subsequent independent measurements on both branches showed how little difference there was between them.

The next step in the analysis was to determine how the rate of fall of pressure varied with the pressure. By means of a Richards-Roope tangent meter the tangents to the curves of figure 2 were measured at intervals of 2 mm. Hg. These rates of pressure fall were then plotted against the pressures at which they were observed, to give figure 3.

We would emphasize three characteristics of the curves of figure 3 as important in drawing conclusions from these experiments.

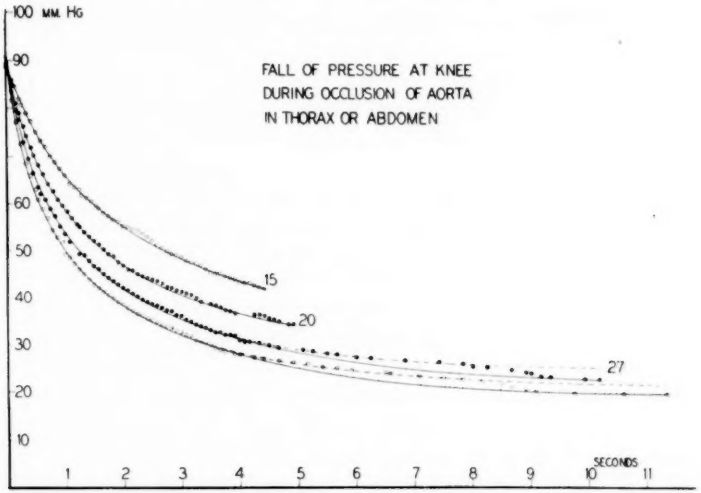

Fig. 2. Pressure-time reconstructions of several records like *K* in figure 1; 15, 20, 27, distances in centimeters up from femoral entrance to point of occlusion.

1. They are not straight lines: i.e., the rate of fall of pressure is not linearly proportional to the pressure, whether the latter is considered as a whole or is diminished by any constant amount.

2. The longest one intersects the pressure axis at about 20 mm. Hg: i.e., at this level the fall of pressure (and therefore, presumably, the outflow from the reservoir) ceases. Four occlusions which were long enough to bring about this equilibrium yielded curves with intercepts in the range from 18 to 26 mm. Hg; shorter ones all showed tendencies favoring a similar extrapolation.

3. With occlusions successively farther up the aorta, the rate of fall of pressure is progressively greater at a given pressure. This appears as a larger amount of "sag" in the curves of figure 2. It should be pointed out

that in no dog did we succeed in demonstrating more than three different curves, even though the aorta was occluded at more than three points. Our evidence suggests the existence of three rather broad regions in the abdominal and lower thoracic aorta, within each of which the outflow is much the same, rather than a smooth continuous variation. One of the four dogs was autopsied to check anatomical landmarks with cannula readings, and the results are suggestive. Plotted as in figure 3, an occlu-

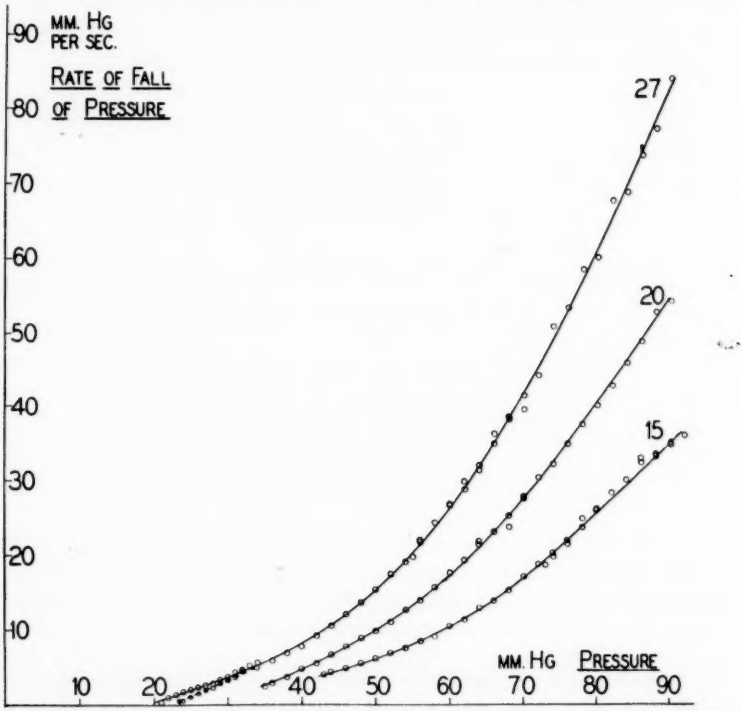

Fig. 3. Rate of fall of curves of figure 2 at each pressure, obtained by plotting tangents; 15, 20, 27, as in figure 2.

sion below the renal arteries gave one curve; between the renals and the coeliac axis, another curve; while occlusion at two different levels above the coeliac axis gave curves which were indistinguishable from each other. Apparently, moving the occlusion up past such large drains included less reservoir capacity in proportion to the additional abdominal channels for outflow. The dotted end of the long curve in figure 3 shows how little difference it makes whether the solid or the dotted end of the curves of figure 2 be measured.

DISCUSSION. The curves of figure 3 represent the resultant of two factors which must first be considered separately: namely, the peripheral resistance and the elastic recoil of the walls of the distributing arteries.

If we were dealing with the streamline flow of a homogeneous liquid through rigid tubes, the variation of flow with pressure would be shown by a straight line rising from the origin with a slope dependent upon the resistance. If, in addition, the volume-elasticity coefficient of the reservoir were constant, the curves of figure 3 should also be straight lines rising from the origin. It is our object to assign the most probable causes for the deviations of the experimental curves from this simple picture—their curvature and their intercepts on the pressure axis.

As Herrick (7) has recently pointed out, it is not yet possible to estimate the part played by each of the factors involved in the resistance which is met by the blood in the small vessels. The tubes are not rigid, the degree of turbulence (if any) has not been determined, and the viscosity of the blood is not the same at different velocities. However there are clues in the literature.

Fåhraeus (3) has shown that the decrease in the apparent viscosity of blood with increasing velocity in very small tubes is due to the concentration of the corpuscles along the axis of the stream. This situation in itself presupposes streamline flow, since any turbulence would break it up and only laminar flow would produce the forces drawing particles to the axial core. (Fåhraeus' explanation of these forces, invoking the Bernoulli principle, is hydraulically unsound. A better explanation would seem to lie in the difference of shear modulus between particles and liquid.)

The closest approach to a direct determination of the pressure-flow relationships in the peripheral vascular bed is the work of Whittaker and Winton (8). They found that in the isolated hind leg of a dog, when conditions were properly stabilized, the flow of blood was linearly proportional to the applied pressure—without regard for the manner in which the various factors might be interacting to produce this condition. In so far as these results may be taken to represent normal constancy of the peripheral resistance over a wide pressure range for short periods of time, the curvature of the lines of our figure 3 must be explained not on the basis of changes in the resistance but rather of variation in the volume-elasticity coefficient of the reservoir—the latter becoming less distensible as it is stretched.

For the apparent cessation of flow in the neighborhood of 20 mm. Hg, indicated by figure 3, various explanations have been offered. In the similar results of Whittaker and Winton (8), venous pressure, tissue pressure, and osmotic pressure were excluded from importance by two findings: 1, the phenomenon occurred in glass viscosimeters as well as in blood vessels; 2, the intercept varied with corpuscular concentration and very nearly

vanished with removal of the red cells. The true explanation in this type of experiment may well lie, as they suggest, in the anomalies of the apparent viscosity of blood. In this concept the viscosity, abnormally high at low velocities in small tubes, reduces the flow at low pressures to an amount which is no longer detectable. The possibility of collateral supply, as suggested by the recent report of Eckstein and Gregg (2), has not been excluded in our experiments. Its existence to support the pressure at 20 mm. Hg was not evident in the form of pulsations in the records measured.

The work of Whittaker and Winton certainly furnishes no support for the belief that a variable quantity must be subtracted from the arterial pressure to get the true driving pressure—a maneuver employed by some analysts to straighten their logarithmic curves.

The determination, from the curves of figure 3, of the exact function of the pressure represented by the volume-elasticity coefficient offers an attractive prospect. Attempts to do it have so far proved fruitless in eliciting any formula which would hold good over the whole range of pressures. This is not surprising in view of the extremely complicated formulation which results from theoretical treatment of the situation, even if the highly simplifying but untrue assumption be made that the tension modulus of the arterial wall is constant. Furthermore, these curves represent the reactions not of individual arteries but of whole sections of the arterial reservoir, composed of many kinds and sizes of vessels.

There have appeared recently three contributions with which the present results should be compared: the work on normal diastoles in many animals by Woodbury and Hamilton (9); the publications by Gomez (4) and by Gomez and Veil (5); and the paper by Eadie and McCrea (1). Where they overlap in the higher pressure regions our results are in accord with those of Woodbury and Hamilton; but at lower pressures they show that these authors were justified in limiting their empirical formulations to pressures in the normal diastolic range.

Gomez, alone or with Veil, has defended three propositions hereto pertinent: 1, analysis of normal diastoles (by plotting the logarithm of the "effective pressure" against time) shows the fall of pressure to be proportional to the pressure and predicts cessation of flow at 45 mm. Hg; 2, pressure actually ceases to fall at 45 mm. Hg when the aorta is occluded or when the heart is stopped by stimulation of a vagus whose infracardiac fibers have been cut; 3, cardiac arrest brought about by stimulation of a vagus whose infracardiac fibers are intact results in a fall of arterial pressure to zero.

In the particular experiment reported here, the upper ends of the curves of figure 3 appear to be almost straight for a short distance. These por-

tions, if prolonged downward, would cut the pressure axis in the neighborhood of 50 mm. Hg. Such observations might seem to confirm the first proposition of Gomez. Our long curves show, however, that the apparent fitting of such results to any formulation over a short range of pressures is quite fortuitous. For the other two Gomez propositions we can show nothing but quite contrary results. No record of ours in which the blood supply was clearly cut off showed any tendency to stop falling at 45 mm. Hg. None of the curves reported here resulted from vagus stimulation; but in other analyses we have been measuring the pressure at the end of many such periods of cardiac arrest—brought about by vagus stimulation or acetyl choline injection—and even after prolonged cardiac inactivity we found only one instance in which the pressure fell below 15 mm. Hg.

In this connection the results reported by Eadie and McCrea (1) appear to agree more closely with those of Gomez and Veil than with ours. However, they do not actually report pressures falling to zero. We believe that our method of plotting tangents instead of logarithms permits a more straightforward presentation of the experimental findings. There is no necessity for determining by trial a quantity to be subtracted from the measured pressure to get the "effective" pressure. There has been abundant experimental demonstration, both direct and indirect, in the last eighty years, that the volume-elasticity coefficients of arteries do change with distention of the vessels. Until enough consistency is found in this variation to justify a general mathematical formulation of it, there can be no "theoretical" form for the curve of fall of pressure in arteries whose central blood supply has been stopped.

SUMMARY

1. Curves are presented which show the course of fall of pressure in an artery at a dog's knee when the aorta is occluded at various levels.

2. By plotting the tangents to these curves, a second set is obtained, showing the rate of fall of pressure as a function of the pressure.

3. Three characteristics of these derived curves are pointed out:

a. They all bend upward with increasing pressure. If Whittaker and Winton's straight pressure-flow lines be accepted, this furnishes additional evidence for an increase in the volume-elasticity coefficient of the arterial reservoir with rising pressure.

b. They (or their extrapolations) intersect the pressure axis in the neighborhood of 20 mm. Hg. This agrees with Whittaker and Winton's finding that, due to anomalies in the apparent viscosity of suspensions, lower pressures than this will not force blood of normal corpuscular concentration through the peripheral vessels at any appreciable rate.

c. Occlusion of the aorta at three different levels seemed to alter the

balance between reservoir capacity and drainage: the higher the occlusion the more rapid the emptying.

4. The pertinent principles of hydrodynamics and elasticity are briefly reviewed and the present results are compared with some reported previously.

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THE VENOUS RETURN AS A FACTOR AFFECTING THE VITAL CAPACITY

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In 1932, Hamilton and Morgan suggested that pulmonary congestion was the chief cause of the diminution in vital capacity associated with the assumption of the supine position. They found that when cuffs were placed around the bases of the four extremities and inflated to approximately diastolic pressure, the postural reduction in vital capacity was nearly abolished. In one of their four subjects they even noticed a slight increase. Without the cuffs all four showed the usual diminution in vital capacity when they lay down. No attempt was made in that study to collect and treat statistically any great number of data on a large group of subjects.

The present analysis takes advantage of a fairly extensive series of determinations on a representative group of medical students. For several years we have used a modification of the Hamilton and Morgan experiment in the medical physiology course (Doctor Hamilton's laboratory manual) as a study in the mechanics of respiration and an exercise in the statistical method. The students carry out the experiment with real enthusiasm and considerable mutual rivalry, and these conditions make us confident of the reliability of the individual figures and the faithfulness with which the work has been done. Each student blows into a spirometer ten times to determine his vital capacity. When this has been done by all members of a group of three, the procedure is repeated with the subject lying on his back on a cot; again, supine, but with cuffs inflated to 70 mm. Hg cutting off the venous return from the arms and legs.

In order to eliminate the fluctuations of the absolute values from one subject to another, the results are treated by the method of differences. Each student's tries are recorded in three columns, side by side, and the individual figures are subtracted to give ten measurements of the difference in vital capacity between standing and supine positions and ten

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measurements of the difference supine with and without the cuffs. These differences are then treated as frequency distributions and the means are compared with their own standard deviations (t criterion) to test the signifi cance of their deviations from zero.

From the last class to do this experiment we were able to secure complete data on 42 students (41 male and 1 female). The mean value of the 420 differences in vital capacity produced by changing from the standing to the recumbent position was 192 cc. The standard deviation of this mean was 10.7 cc., giving the highly significant t value of 18.0.

When the figures from supine subjects with and without cuffs were compared, it was found that the application and inflation of the cuffs increased the vital capacity, but not as much as would a change to the standing position. The mean value of the differences in this case was only 102 cc., with a standard deviation of 11.9 cc., giving another highly significant t of 8.6.

Four of the students gave quite anomalous figures, with (for reasons unknown) either no change or an actual increase in vital capacity when they lay down. When these four were left out of the series, the figures naturally showed less variation. For the remaining 38, the mean value of the reduction of vital capacity with recumbency was 223 cc., with this mean showing a standard deviation of 9.5 cc. ($t = 23.4$). The mean value of the increase in vital capacity with "bloodless venesection" was 140 cc. The standard deviation of the mean was 9.6 cc. ($t = 14.5$).

These ratios of the means to their standard deviations (t values) are sufficiently high to make the deviations of the means, not only from zero but also from each other, so highly significant as to be practically indubitable, particularly with samples of such large size. The reduction in vital capacity with recumbency has been known for a long time (for references, see Hamilton and Morgan's paper). It now appears that the increased venous return and the resulting accumulation of blood in the pulmonary vessels may account for somewhat more than half of this diminution. Certainly, this fraction of the reduction is not observed when the blood is prevented from returning from the extremities which are dependent when the subject is standing.

SUMMARY

Thirty determinations on each of 42 medical students show, on statistical analysis, the following result: the reduction in vital capacity commonly observed to take place when a person changes from the erect to the supine position is less than half as great if blood is prevented from returning to the thorax by means of diastolic-pressure cuffs about the bases of the four extremities.

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